University of Alberta

Feedback interactions between forest tent caterpillars (*Malacosoma disstria)* and trembling aspen (*Populus tremuloides)* in central Alberta, Canada

by

Andrea Durand ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science**

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Feedback interactions between forest tent caterpillars (*Malacosoma disstria*) and trembling aspen (*Populus tremuloides*) in central Alberta, Canada** submitted by **Andrea Durand** in partial fulfillment of the requirements for the degree of Master of Science in Environmental Biology and Ecology.

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Abstract

Positive and negative feedback interactions between forest tent caterpillars (*Malacosoma disstria* Hbn.) and trembling aspen (*Populus tremuloides* Michx.) may play an important role in the performance of this insect and perhaps even contribute to its cyclic population dynamics. Early spring larval feeding on opening buds did not induce strong rapid changes in the leaf quality of aspen; instead, some evidence for negative impact on caterpillar growth and development was found. Real and simulated insect defoliation in early summer (mid June) induced foliar changes in trembling aspen that had negative effects on larval performance. These changes were induced systemically throughout the tree and occurred a year following the damage. Significant interclonal variation in response to insect feeding damage was found, despite inherent clonal differences in leaf quality. Furthermore, true insect feeding appeared to elicit stronger induced defenses than mechanical wounding. This suggests that induced responses are indeed defensive. Whether or not they evolved as responses to herbivores in general or are co-evolutionarily linked specifically to *M. disstria*, remains to be determined.

To my parents, Karin and Paul, who taught me to appreciate and explore the wonders of the natural world.

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Chapter 1

Introduction

1.1 *Malacosoma disstria* Hübner

The genus *Malacosoma*, the tent caterpillars, includes 26 described species that are restricted to the temperate regions of the Northern Hemisphere (Holarctic region). Twenty of these are old world species (Palearctic region), while the remaining 6 are found in the new world (Nearctic region) (Fitzgerald 1995). Of the North American species, the forest tent caterpillar *M. disstria* Hübner (Lepidoptera: Lasiocampidae) has the widest distribution, occurring throughout most of the United States, Canada and likely also into Mexico, wherever deciduous trees are found naturally (Stehr and Cook 1968). In contrast to other tent caterpillars, *M. disstria* larvae do not construct tents; they spin silken trails and mats on the foliage, twigs and large branches, where they rest between feeding periods (Stehr and Cook 1968, Fitzgerald 1995).

The life cycle of the forest tent caterpillar is, in most respects, typical of other *Malacosoma* species. Adult females lay all of their eggs (on average 150 to 200) in a single helical band around small twigs (Ives and Wong 1988). Eggs are then covered with a frothy substance called spumaline, produced in the female's accessory gland (Hodson and Weinman 1945). Embryogenesis occurs soon after the eggs are laid, and within four weeks fully developed caterpillars are formed (Ives and Wong 1988). The pharate first instars remain inside the

eggs until the following spring, because exposure to low winter temperatures is required to break the obligatory larval diapause (Fitzgerald 1995).

Forest tent caterpillars usually synchronize hatching with the leaf flush of their host tree and begin feeding in early spring. First instars chew their way out of the eggs and remain on the surface of the egg mass until most of their siblings have eclosed (Fitzgerald 1995). They forage nomadically, with groups of larvae frequently moving to new and distant locations in search of food (Fitzgerald and Peterson 1988). Only when the fourth or fifth larval stadium is reached, do caterpillars begin to disperse and feed independently. At the same time, they become less discriminating about what host plant they feed on. A wide variety of deciduous tree species have thus been listed as hosts for this insect, even though egg bands are never laid on them (Stehr and Cook 1968).

Five to six weeks after first emerging from their eggs, larvae spin a cocoon and pupate in the remaining uneaten foliage or in other protected areas if the trees are stripped completely of their leaves. Moths emerge from cocoons approximately 10 days later (Ives and Wong 1988). Mating usually occurs within hours of female eclosion and the gravid females then disperse to find suitable oviposition sites (Fitzgerald 1995). Adults, having no functional mouthparts, do not feed and die within approximately two days (Stehr and Cook 1968).

1.2 *Populus tremuloides* Michaux

The main host of forest tent caterpillar in Canada is trembling or quaking aspen, *Populus tremuloides* Michaux (Ives and Wong 1988). The genus *Populus* belongs to the willow family, Salicaceae, and includes 6 native species in Canada (Farrar 1995). Trembling aspen is the most widely distributed tree species in North America (Peterson and Peterson 1992). Its range spans almost the entire continent, excluding a narrow coastal band of British Columbia and Alaska. Significant stands of aspen can be found as far north as the Mackenzie River delta of the Northwest Territories and as far south as Catron County, New Mexico (Little 1971). However, sparse distributions of this tree species occur as south as 21°N in Mexico, in the western part of its range (Little 1971).

The economic importance of hardwood species like trembling aspen has risen steadily since the 1980s, owing to the growing scarcity of accessible softwood timber in Canada (Ondro 1989). Of Alberta's total timber harvest in 1980 only 2.4% consisted of aspen; this increased to 17.2% in 1988 (Alberta Energy and Natural Resources 1982-86, Alberta Forestry, Lands and Wildlife 1988). Today, aspen is considered an excellent raw material for pulp, paper, waferboard and oriented strandboard (OSB) (see Peterson and Peterson 1992).

Although aspen is a prolific seed producer, most stands are regenerated asexually through root suckering. As a result, each genetically distinct individual or 'clone' is composed of many separate woody stems called ramets (Barnes 1966). Clone size varies dramatically in different regions of North America. In

the northern mixedwood regions of Canada and the eastern United States, clones tend to occupy relatively small areas, ranging in average from 0.006 to 0.08 hectares (Barnes 1969, Steneker 1973). In contrast, clones along the southern Rocky Mountains (e.g., Sevier County, Utah) occupy up to 43 hectares and may consist of up to 47,000 ramets with an average DBH of 280 mm (Kemperman and Barnes 1976). Trembling aspen exhibits extensive interclonal variation in traits like leaf morphology (Barnes 1969), growth rates (Zahner and Crawford 1965), time of leaf flush and senescence (Barnes 1969), susceptibility to damage by disease and frost (Barnes 1959), and resistance to insects (Chilcote et al. 1992, Hwang and Lindroth 1997).

1.3 Forest tent caterpillar - trembling aspen system

The forest tent caterpillar is the most destructive defoliator of trembling aspen in the Canadian prairie provinces (Ives and Wong 1988). Population outbreaks of this insect are 'cyclic' in aspen stands. They recur at intervals of 6 to 16 years, with an average of 10 years (Hildahl and Reeks 1960), and often last from three to four consecutive years (Duncan and Hodson 1958, Witter 1979). Outbreaks have even been found to last up to nine years (Sippell 1962). These extreme population increases cause severe and extensive defoliation. From 1988-1992 the total area of aspen defoliation in the prairie provinces often amounted to millions of hectares annually (Brandt 1995). In Alberta, growth losses due to forest tent caterpillar defoliation was estimated to be about

10,300,000 m^3 between 1982 and 1987 (Brandt and Amirault 1994). This represents a 13.5% growth volume loss of the total aspen growth in Alberta over this period.

1.4 Thesis Objectives

Despite considerable research efforts, the proximate causes for the onset and termination of forest tent caterpillar outbreaks remain somewhat elusive. The importance of density-independent factors such as weather (Blais et al. 1955, Hodson 1962, Witter et al. 1975), and density-dependent factors, such as natural enemies (Hodson 1941, Witter et al. 1972, Parry et al. 1997), have been investigated. However, to fully understand such extreme fluctuations in the abundance of a herbivore population, the effects of host quality must also be considered. In studies with other species, notably Haukioja's work on the autumnal moth-mountain birch system (e.g., Haukioja and Hakala 1975, Haukioja 1980, Haukioja and Neuvonen 1985, Haukioja et al. 1990), the effects of variable food quality have been found to play an important role in regulating population dynamics of the insect herbivore (see also Baltensweiler et al. 1977, Benz 1974, 1977).

The primary objective of this thesis is therefore to investigate the possibility that similar interactions occur between forest tent caterpillars and trembling aspen. More specifically, does feeding damage by forest tent caterpillars alter the leaf quality of *P. tremuloides*, so as to significantly influence

the performance of the current and/or subsequent herbivore generation? Each of the following two chapters in this thesis addresses a different aspect of this relationship.

In chapter 2, I ask whether a significant amount of forest tent caterpillar feeding damage to newly flushing and unfurling leaves in spring can improve the foliage quality of aspen ramets for *M. disstria* larvae, either in the current or following year of damage. These types of interactions have been reported for other host-defoliator interactions (Tallamy 1985, Dussourd and Eisner 1987, Haukioja et al. 1990, Sagers 1992, Senn and Haukioja 1994) and in some cases such positive feedback could allow the insect herbivore to build to epidemic levels (Haukioja et al. 1990, Senn and Haukioja 1994). I test for this relationship by assessing caterpillar performance on foliage from trees that had opening buds removed in spring and those which did not.

In chapter 3, I then examine the effects of severe later-instar larval damage to fully expanded leaves in mid June on the leaf quality of aspen ramets a year following defoliation. Here, the implication is that if foliage quality declines significantly then delayed negative feedback may be introduced into the population dynamics of the herbivore. This type of plant-herbivore interaction is thought to be a main contributing factor to the collapse of certain forest lepidoptera outbreaks (Benz 1974, 1977, Haukioja and Hakala 1975, Baltensweiler et al. 1977). Furthermore, I test whether such induced changes in leaf quality are responses to pressures exerted specifically by insect herbivores. If so, foliage quality for larvae should be reduced more following natural insect

damage than mechanical wounding (Haukioja and Neuvonen 1985). In chapter 2 and 3, I also assess aspen's interclonal variation in response to insect damage and overall resistance to forest tent caterpillars.

Finally, chapter 4 summarizes and discusses the importance of my findings in the context of other relevant work pertaining to the population dynamics of *M. disstria*.

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Chapter 2

Forest tent caterpillar performance on trembling aspen clones after spring removal of flushing buds

2.1 SYNOPSIS

This chapter examines whether foliage quality of trembling aspen (*Populus tremuloides* Michaux) improves for forest tent caterpillar larvae after the removal of apical buds in the spring at the time of leaf flush. In 1997, ramets from four aspen clones had approximately 25% of their most apical flushing buds destroyed by manual clipping only; in 1998, trees from three of the four clones had buds removed by either first instar forest tent caterpillars (*Malacosoma disstria* Hübner) or clipping. Leaf quality was assessed in 1998 by bioassays using fourth instars and by measures of leaf size. Measures of larval performance proved to be much better indicators of changes in leaf quality than leaf area. In general, caterpillars performed equally well on previously clipped/mined ramets and controls. However, larvae from clone 1, reared from the leaves of ramets artificially clipped in the current year (1998), took significantly longer to develop, had lower relative consumption rates, and were less efficient at converting digested and ingested food into biomass than those from control trees. This suggests that rapid defenses were induced in the same season as the damage. These foliar changes were short-term, not lasting beyond the year of damage.

2.2 INTRODUCTION

Plants respond variably to insect feeding (Karban 1987, Coleman and Jones 1991, Haukioja and Honkanen 1996). For example, true and simulated herbivore damage can induce foliar changes that either affects herbivores adversely via induced defenses (Benz 1974, 1977, Haukioja and Hakala 1975, Baltensweiler et al. 1977, Clausen et al. 1989, 1991), positively via induced amelioration (Myers and Williams 1984, Danell and Huss-Danell 1985, Roland and Myers 1987, Haukioja et al. 1990), or not at all (Neuvonen et al. 1988, Hanhimäki and Senn 1992). The direction of this response may in large part be determined by the timing of the damage and the type of tissue fed upon (Danell and Huss-Danell 1985, Neuvonen et al. 1988, Haukioja et al. 1990).

In mountain birch (*Betula pubescens*), the wounding of expanding or newly matured leaves in late spring and early summer causes leaf quality to deteriorate for herbivores in both the current (Wratten et al. 1984, Haukioja and Hanhimäki 1985) and following year (Haukioja and Neuvonen 1985, Neuvonen et al. 1987, Ruohomäki et al. 1992). Conversely, clipping or mammal browsing of dormant apical buds and/or twigs in the winter, results in the production of larger, more nitrogen- and chlorophyll-rich leaves in the following growing season (Danell and Huss-Danell 1985, Danell et al. 1985, Senn and Haukioja 1994). These previously moose-browsed mountain birches were also attacked by greater numbers of leaf-eating and sucking insects (Danell and Huss-Danell 1985). Furthermore, *Epirrita autumnata* (Lep., Geometridae) larvae had higher

relative growth rates on trees with the apical buds removed in winter (Haukioja et al. 1990).

Similar to the effects of winter moose browsing, insects that damage developing apical buds or unfurling leaves in the spring may also induce inceases in leaf quality (Senn and Haukioja 1994). Insect removal of apical meristematic tissue either (a) disrupts apical dominance, which increases the resource status of the previously suppressed basal buds, or (b) simply reduces the number of buds which compete for limited nutrients (Haukioja et al. 1990, Bryant et al. 1991, Honkanen et al. 1994, Honkanen and Haukioja 1994). In this way, foliar quality of the remaining buds may improve. Naturally, in the above case (b), an ameliorating effect would only occur if the plant fails to compensate for the loss of these buds by activating or producing other meristematic units (Bryant et al. 1991).

Forest tent caterpillars (*Malacosoma disstria*), being classic early season defoliators, also feed gregariously on opening buds and unfurling leaves growing at the apex of branches (Hildahl and Campbell 1975, Durand personal observation). In this chapter I test whether true or simulated early larval feeding damage, executed in early spring, induces aspen to ameliorate the quality of their remaining leaves. To accomplish this, leaf quality is assessed indirectly through bioassays and measures of leaf area. I further address the question of whether aspen ramets compensate for the loss of their growing buds by activating latent buds. Induced amelioration at the onset of an insect outbreak is a positive feedback that could initiate or enhance population growth (Haukioja et al. 1990).

Induced defenses could, by contrast, dampen such population growth (Haukioja and Neuvonen 1985), and if induction is delayed, time lags could contribute to population cycles (Haukioja 1980).

2.3 METHODS

2.3.1 Site description and clone selection

The study was conducted between 1997 and 1998, at the George Lake Field Station (114°06'W, 53°57'N), approximately 75 km northwest of Edmonton, in central Alberta, Canada. The forest was dominated by mature to over-mature trembling aspen (*Populus tremuloides* Michx.), which had experienced moderate to severe defoliation by forest tent caterpillar throughout most of the 1980s (Parry, Spence and Volney 1997). By 1996, new aspen saplings representing various clones had become well established in the gaps of the more decadent stands.

Although forest tent caterpillar egg bands and small larvae are found predominantly in the overstory trees of a forest (Batzer et al. 1995), for practical reasons, my study was limited to test the effects of bud removal on small understory trees. Given the resources at hand, manipulating bud numbers of mature aspen was logistically impossible. I first identified ramets of the different aspen clones by observing leaf flush times in the spring and leaf structure (i.e., shape once fully expanded) (criteria outlined by Barnes 1969, Steneker and Wall 1970). Clones selected for study had at least 6 ramets and met the following

requirements: 1) suitable size (height = 3 – 5 m; diameter at breast height = 15 – 25 mm); 2) a shape free of major crooks in the main stem; 3) free of disease symptoms and hare/deer browsing damage; 4) growing in the open or in partial shade. Another criteria for clone selection was that they be in close relative proximity to one another, to minimize site variations (Fig. 2.1). Four suitable clones were found and mapped in the upland aspen forest (see Niemelä et al. 1992 for detailed description of the forest type). Tree ring counts, made at the end of the experiment, indicated that the ramets were 10-12 years old. Thus the trees used in this study may have been previously defoliated as very young shoots because the last forest tent caterpillar outbreak at George Lake did not collapse until 1989.

2.3.2 Bud removal treatments

Prior to implementing the bud removal treatments, the number of active buds (i.e., those resulting in shoots) on each ramet was recorded so that the number of developing buds required to achieve a 25% loss could be determined. Development of the aspen buds was monitored, and when a clone began to show obvious signs of bud expansion, the phenological stage of each bud was classified according to Parry, Volney, and Currie (1997). Buds that were in the 2nd (i.e., green coloration visible along edges of the bud scales) to 4th (i.e., scales separating, leaf tips visible) condition class were classified as active, while those still in the 1st condition class (i.e., no apparent development) were classified as dead.

Buds were destroyed on each clone at the time of budbreak, when the majority of buds on the ramets were in the 5th (i.e., scales separating, leaf tips protruding and elongating) and 6th condition class (i.e., bud scales shed, leaves elongating and unrolling) (Parry, Volney, and Currie 1997). This damaged the trees at a time when larvae would naturally begin feeding. A larger proportion of the most apical buds (≤ 4 buds / branch) were removed from the top branches of each designated ramet. No buds were removed from branches shorter than 12 cm. These protocols were followed because egg masses of natural outbreaks are primarily found at the tip of branches (Fig. 2.2 a), and in the upper part of the canopy (Shepherd and Brown 1971).

One type of control and two types of bud removal treatments were used in this study. These three treatments were randomly assigned to ramets and replicated twice (3 x 2 = 6 ramets) within each clone. In the first bud removal treatment, opening buds were damaged by forest tent caterpillars (natural bud removal). Surface sterilized egg bands were attached with 'twist tie' wires near buds that were subject for removal; then they were enclosed with a 16 x 12 cm bag made of No-see-um netting (Totem Outfitters, Edmonton, AB). Only egg masses from which larvae had already begun to emerge were used for mining and feeding on the buds. They were placed on the trees when the majority of the buds were in the 4th condition class, because this allowed enough time for all the larvae to hatch, feed and kill buds that had developed into the 5th and 6th condition class. In warm or typical weather conditions, forest tent caterpillar emergence is usually well synchronized with budbreak of their host tree (Ives and

Wong 1988, Parry et al. 1998). Insects were allowed to feed for 3 to 4 days until all of the unfurling leaves were consumed. In the second removal treatment, buds were removed by clipping them at their base with pruning shears (artificial bud removal). Again buds were destroyed when they had reached the 5^{th} or 6^{th} condition class. No buds were removed from control ramets.

I removed 25% of the opening buds of the manipulated aspen ramets. A loss of this magnitude was used for my experiment to simulate natural conditions [1.2 egg bands per upper branch results in moderate to severe defoliation by forest tent caterpillar (Shepherd and Brown 1971)]. The total number of branches on each of my experimental ramets was multiplied by 1.2 to arrive at the number of egg masses that would cause moderate to severe defoliation. I assumed that the larvae from one egg mass would be able to destroy one developing bud because the first larval stadium only accounts for 0.23% of the total foliage consumed during the lifetime of a forest tent caterpillar (Hodson 1941). The calculated number of egg bands for each ramet (see above) would therefore amount to a loss of approximately 25% of the total number of active buds on any given ramet.

The two methods of bud removal were equally effective at destroying approximately 25% of the developing buds. However, manual clipping killed buds considerably faster than caterpillar feeding.

The natural bud removal treatment was attempted in 1997, but failed in all clones because egg bands, collected the previous autumn from a moderate outbreak near Bonnyville, AB, did not hatch. For this reason, the natural and

artificial bud removal treatments were repeated in the spring of 1998. The trees that were intended for the natural bud removal treatment in 1997 were again assigned to the same treatment. Similarly, control ramets remained as controls in both years, however, new trees were selected for the 1998 artificial bud removal treatment. Finally, a fourth treatment of 1998 were ramets previously clipped in 1997 but not manipulated in 1998.

An insufficient number of suitable clone 2 ramets were available for treatment in 1998 because of deer browsing in the winter. For this reason data from clone 2 was removed from the analysis in 1998.

2.3.3 Assessment of bud/shoot mortality and compensatory growth

Compensatory growth is brought about by activation and growth of latent buds (see Fig. 2.2 b) after significant plant tissue loss. Thus to determine whether trembling aspen ramets show compensatory growth (see Fig. 2.3) following the removal of approximately 25% of the apical and basal meristems, the number of active and latent buds before and after the treatments had to be counted. Detailed sketches of the branches prior to the implementation of the treatments were made. These 'bud maps' included the location of a) active, dead and latent buds, with respect to both each other and the internodes; b) the location of active buds selected to be destroyed on the treated trees. To determine the fate of both active and latent buds, trees were reassessed at the end of the summer by counting the number of live shoots and latent buds. The amount of background and total (background plus treatment induced) bud/shoot

mortality could be calculated along with the amount of compensatory growth (i.e., number of activated latent buds). Assessment of a ramet's bud/shoot population was made on control and artificially clipped trees in 1997 and on artificially clipped and naturally mined trees in the following year. For logistical reasons, control ramets were not reassessed in 1998.

2.3.4 Assessment of leaf quality

First, leaf quality was assessed through bioassays using fourth instar forest tent caterpillars. Although this method does not indicate what specific plant quality has been altered by the treatment, bioassays that use whole leaves have the advantage of actually measuring the impacts of foliar changes on the target insect (Myers 1988, Neuvonen and Haukioja 1991). The second method of assessing leaf quality was by comparing leaf size of treated and untreated trees. This method allows for comparisons with previous studies of induced amelioration, that often rely heavily on measures of leaf morphology in determining changes in host quality (Danell and Huss-Danell 1985, Danell et al. 1985, Haukioja et al. 1990, Senn and Haukioja 1994). Increased leaf size in mountain birch offers a good indication that quality had improved: larger leaves are usually darker green, heavier, more nitrogen-rich, and are preferred by other insects (Danell et al. 1985, Danell and Huss-Danell 1985, Haukioja et al. 1990). Furthermore, in large aspen tortrix (*Choristoneura conflictana*), pupal mass varies directly with leaf area of trembling aspen [i.e., pupal mass of *C. conflictana* = 108 + 2.8 (area), r = 0.924] (Clausen et al. 1991).

Fourth instar larval feeding trials

The larvae used for bioassays and the natural bud removal treatment in 1998, were obtained from egg bands collected from a young outbreak near Flin Flon, Manitoba in October, 1997. They were kept over-winter in large brown paper bags at -10° C until April 1998. Only large, regular shaped egg bands with complete spumuline coverings were selected to produce insects for the bioassays. Spumuline was removed from egg bands by scraping and surface sterilized according to the procedure outlined by Parry, Spence and Volney (1997) to prevent infection by nuclear polyhedrosis virus (NPV).

Larvae from some egg masses were used to mine the buds. The remaining larvae were combined and reared on the same field-collected foliage until they reached the end of the third stadium. Newly molted fourth instars were weighed and used for feeding trials that began in late May, 1998. Caterpillars were reared individually in 150-ml wax paper cups (Lily Cups Inc., Scarborough, ON) at a constant temperature of 25° C, under a 16 : 8 (L : D) photoperiod. Fourth instars were randomly assigned foliage from one of the experimental ramets for the duration of the feeding trial. A total of 20 larvae were reared on foliage from each ramet (20 x 8 x 4 = 640). The leaves they received came from both the top and bottom of the canopy and were first weighed to obtain wet leaf masses for foliage biomass determinations. Petioles of leaves were wrapped in cotton and placed in small glass vials of water to keep them fresh. Foliage was replaced every 2 days until larvae completed the fourth stadium, at which point

the larvae were frozen. Cups were checked for new fifth instars every 2 hours except between 12 midnight and 6 am. Feeding trials were terminated after 7.5 days because foliage on some experimental trees had been exhausted. Caterpillars that failed to molt within that time were frozen and used for calculations of nutritional indices. Development time for each larva was calculated in days.

Caterpillars were not sexed for these bioassays because other work with this population indicated that growth and feeding efficiency indices for males and females did not differ significantly (see Chapter 3). Larvae, frass and uneaten leaves were dried (at 50° C for 48 hrs) and weighed. Standard nutritional indices were calculated for each larva, including relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested (ECD) and ingested (ECI) food (Scriber and Slansky 1981, Waldbauer 1968) (see Appendix 1 for calculations of nutritional indices). The initial dry mass of leaves were estimated from regression analysis of the wet to dry mass ratio of variably sized leaves from the four clones ($y = 0.33 x + 8.55$, $n=315$, $P < 0.001$, $r^2=0.96$). One hundred newly molted fourth instar larvae, that were sacrificed before the feeding trails began, were also used to develop regression estimators of initial dry mass based on the live mass of individuals ($y = 0.134 x - 0.117$, $n = 100$, $P < 0.001$, $r^2 = 0.93$).

Determining leaf size

Leaf area of the experimental trees could not be measured directly, as it was undesirable to remove leaves from these trees. Instead, leaf area was estimated from regression analysis of the leaf width to leaf area ratio of variably sized leaves. Trembling aspen have two types of shoots: heterophyllous or long shoots, that continue to elongate through summer (7 or more leaves); and preformed or short shoots, that produce a fixed number of leaves in spring (1 to 6 leaves) (Peterson and Peterson 1992). Regression equations were thus derived from random samples of each shoot type, taken from four non-experimental ramets in each of the four clones (with the exception of clone 3, for which only one non-experimental ramet was available). Leaf area was measured with a CI-202 Portable Leaf Area Meter (CID Inc., Vancouver, WA). Each leaf was scanned 4 times and the average area was recorded. An analysis of variance (ANOVA) was used to test the effects of clonal origin of foliage, shoot type, leaf length and width on leaf area (Appendix 2 a) (PROC GLM, SAS Institute 1996). This indicated that clone, leaf length and width were all significant contributors to variation in leaf area ($P < 0.001$), but shoot type was not ($P = 0.921$). As a result, four separate regression equations (modified from Cochran 1977, p. 189) were developed for each of the four aspen clones (Appendix 2 b) (PROC REG, SAS Institute 1996). Leaf width, rather than length, was used to predict leaf area because it explained more of the variance in leaf area and because inclusion of both variables required more complex calculations.

In early August 1997 and 1998, leaf width measurements of the experimental ramets were taken from one randomly chosen leaf from each short shoot and four randomly chosen leaves from each long shoot. The average leaf width per tree was calculated (Appendix 3). Each mean was then used in the appropriate regression equation so that leaf area per ramet could be determined (Cochran 1977).

2.3.5 Statistical analysis

Growth responses of artificially clipped, naturally mined and unmanipulated control trees were analyzed by the Mann-Whitney test (Zar 1984).

A two-way analysis of variance (ANOVA) was used to test the effects of clone and the bud removal treatments on instar duration, RGR, RCR, AD, ECD and ECI (PROC GLM, SAS Institute 1996). For each significant main effect and interaction, pre-planned pairwise comparisons, with sequential Bonferroni adjustments to maintain experiment-wide Type I errors at 0.05, were made to ascertain the pattern of difference among least square means (Rice 1989). Comparisons of a priori interest were larval performance 1) among the three clones; 2) among the four treatments; 3) between control trees and artificially clipped ramets of 1997 by clone; 4) between control trees and artificially clipped ramets of 1998 by clone; 5) between control trees and naturally mined ramets of 1998 by clone; and 6) between artificial clipped trees and naturally mined ramets of 1998 by clone.

The effects of the clonal origin of foliage and the bud removal treatments on aspen leaf size was also determined with an ANOVA. Data from 1997 and 1998 were analyzed separately. The same pre-planned pairwise comparisons outlined above were made between the least square means of significant effects.

2.4 RESULTS

Assessment of the bud/shoot population of particular ramets over a growing season, indicated that neither natural nor artificial bud removal treatments further enhanced background bud/shoot mortality later in the growing season. There were no significant differences in the percent background bud/shoot mortality between control and artificially clipped trees in 1997, and artificially clipped and naturally mined trees in 1998 (Table 2.1). However, the total bud/shoot mortality over a summer was significantly greater for artificially clipped trees than for controls; manually clipped and larval mined trees lost approximately equal numbers of buds/shoots (Table 2.1).

Aspen trees did not show compensatory growth, despite having lost approximately 25% of their expanding buds in the spring. Although latent buds were occasionally activated to develop into new shoots (Fig. 2.3), this did not occur significantly more often on clipped than on non-clipped ramets (Table 2.1). Similarly, differences in this response were not detected between trees from the artificial and natural bud removal treatments (Table 2.1). Because ramets lacked substantial compensatory growth after bud removal, they had significantly greater

overall reductions in the number of live shoots from the start to the end of the summer than did control trees (Table 2.1).

The dramatic reduction in the number of expanding buds of an aspen tree appeared to have significant effects on forest tent caterpillar performance in the overall experiment (Table 2.2). Instar duration, relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested (ECD) and ingested food (ECI) all varied significantly with bud removal treatment. The one exception was relative growth rate (RGR), which showed no significant differences (P = 0.485). These performance measures were based on results from successfully molted and non-molted larvae. The non-molted individuals comprised 36% of the total larvae in clone 1. Caterpillars feeding on foliage from clone 2, 3 and 4 all molted prior to termination of the experiment.

Comparisons among least square means of the bud removal treatments and controls revealed that leaf quality did not improve as predicted by the amelioration hypothesis, rather it remained the same or declined somewhat following the loss of expanding buds. Apparent changes in leaf quality occurred between the natural bud removal treatment and the control. Insects in the former treatment, had significantly higher ECD than larvae from the controls (P = 0.003, Fig. 2.4 e), but they had significantly lower AD and therefore failed to increase their RGR above those on the unmanipulated trees (Figs. 2.4 b and d). Furthermore, larval development time was significantly longer for insects on the artificially clipped trees of 1998 than for those on controls (P = 0.006, Fig. 2.4 a); however, the magnitude of difference was only 0.39 days, which is unlikely to be

biologically significant. Leaf quality also appeared to decline for larvae from the artificial bud removal treatment of 1997 because larval consumption rates and approximate digestibility of leaves increased in a way that achieved similar RGRs to larvae reared on the controls (Figs. 2.4 b, c and d). This, along with the failure of larval development times to increase over those of the control group (Fig. 2.4 a), indicated that caterpillars feeding on the artificially clipped trees of 1997 were not actually disadvantaged relative to individuals from the control, even if leaf quality was reduced slightly. If, however, *M. disstria* larvae were reared for their entire larval duration on trees previously damaged in spring, then this could compound the above adverse effects, causing further reductions in their performance.

Differences among aspen clones explained more of the variability in forest tent caterpillar performance, than did the main effect of treatment. Instar development time and nutrition indices of fourth instars fed leaves from the three aspen clones differed significantly (Table 2.2, Fig. 2.5). Larvae feeding on clone 1 consistently performed more poorly than caterpillars on other clones (Figs. 2.5 a, b, c, e and f). For instance, caterpillars fed leaves from clone 1 took at least 2.12 days longer to complete development of the fourth stadium (Fig. 2.5 a) and they had 0.96 mg/mg/day lower relative growth rate (Fig. 2.5 b) than larvae reared on clone 3 or 4. Larvae reared on foliage from clone 1 also had significantly lower consumption rates (Fig. 2.5 c), and a reduced ability to convert digested (Fig. 2.5 e) and ingested (Fig. 2.5 f) food into biomass. On the other hand, insects performed well on both clone 3 and 4 (Fig. 2.5).

Leaf area also differed significantly among clones in 1997 and 1998, however, the bud removal treatments as well as the interaction between treatments and clones had no affect on leaf size neither in 1997 nor in 1998 (Table 2.3).

Comparisons among the clones' least square means of average leaf size in 1998 showed the same trends as the larval performance data. Average leaf area was at least 3.1 cm^2 smaller in clone 1 than in clone 3 and 4 (Fig. 2.6 b). An even greater difference in leaf area was found between clone 2 and 1 in 1997. Leaves in clone 1 were 5.7 cm^2 smaller than leaves in clone 2 ($P < 0.001$, Fig. 2.6 a). Unlike the 1998 data, leaf size did not differ significantly between clone 1 and 4 in 1997 after sequential Bonferroni adjustments of the P values (Fig. 2.6 a).

Significant effects on the insect performance measures of fourth instars were not only attributed to the main effect of treatment and clone, but also to the interaction between defoliation treatments and clones (Table 2.2). For this reason, treatment effects must also be discussed with respect to each clone separately.

The most dramatic effects of the bud removal treatment were seen in clone 1. Larvae feeding on this clone were negatively, rather than positively, affected by the treatments. The foliage from the artificially clipped trees of 1998, was particularly unpalatable for the caterpillars. They took 1.37 days longer ($P < 0.001$) to complete development of the fourth stadium than individuals from the control ramets (Fig. 2.7 b). Insects fed from the artificially clipped ramets (1998) of clone 1 also had significantly lower consumption rates, and were less efficient

at converting digested and ingested food into biomass (Figs. 2.9 b, 2.11 b and 2.12 b). Furthermore, the RGR for larvae feeding on ramets from the artificial bud removal treatment (1998) was 0.26 mg/mg/day lower than for those on control ramets (Fig. 2.8 b). However, this effect became non-significant following sequential Bonferroni adjustments of the *P* value. Finally, the approximate digestibility of leaves from the naturally mined trees in clone 1 was also significantly lower than for those from unmanipulated trees (Fig. 2.10 c).

Leaf quality also appeared to decline for larvae reared on clone 3 and 4 after bud removal. However, these responses were weaker and less consistent than in clone 1. Fourth instars fed leaves from manually clipped (1997) ramets of clone 4 had significantly higher RCR and AD, yet still had similar RGR to individuals fed leaves from control trees (Figs. 2.8 a, 2.9 a and 2.10 a). Contrary to this, larvae reared on trees of clone 4 that were artificially clipped in the current year (1998) had 0.25 mg/mg/day greater RGRs than caterpillars reared on control leaves ($P = 0.002$, Fig. 2.8 b). Only a few larval performance measures in clone 3 varied significantly between ramets that had buds removed and those that did not. Leaf quality in this clone appeared to decline in ramets that were manually clipped the year before because larvae fed from these trees had to consume 4.06 mg/mg/day more leaf tissue than those on unmanipulated trees ($P < 0.001$, Fig. 2.9 a) in order to attain similar RGRs (Fig. 2.8 a). Insects from naturally mined trees of clone 3 had significantly higher ECI than individuals from controls ($P = 0.003$, Fig. 2.12 c), but this again failed to result in significantly higher RGR from the control group.

Overall insect performance measures varied significantly between artificial and natural bud removal treatments of 1998. Development times were significantly longer, while ECD and ECI were significantly lower for larvae reared on the artificially clipped trees than for individuals reared on the naturally mined ramets (Figs. 2.4 a, e and f).

Larval performance also differed significantly between the natural and artificial bud removal treatments when larvae fed on particular clones. Only larvae reared on clone 1, responded variably to the two types of bud removal treatments. Caterpillars feeding on artificially clipped ramets required 1.2 days longer to complete development of the fourth stadium than those on naturally mined trees ($P < 0.001$, Fig. 2.7 d). The ECD and ECI were also dramatically lower for insects reared on the artificial bud removal treatment of clone 1 than larvae on the natural treatment of clone 1 (Figs. 2.11 d and 2.12 d).

Finally, differences between the artificial and natural bud removal treatments can be attributed to either the manner in which buds were destroyed, the effects of the bags, or both. Previous work in the same study area by Parry et al. (1998) does seem to indicate that "bag effects" remain negligible through the early part of May, becoming significant only thereafter with the onset of warmer spring temperatures. This was found even when mesh sleeves remain on the branch for one week (recall that the bags in my study only stayed on the tree for 3 to 4 days).

2.5 DISCUSSION

The overall leaf quality in trembling aspen did not improve for forest tent caterpillar after ramets had approximately 25% of their expanding buds removed. Rather, it remained the same and even deteriorated significantly in some cases (Fig. 2.4). Similary, Kaitaniemi et al. (1997) found that *E. autumnata* larvae fed foliage from mountain birch ramets that had their flushing apical buds removed in the spring by larvae, yielded the same or lower pupal masses than individuals from control ramets. In contrast to this, Senn and Haukioja (1994) reported general improvements in the leaf quality (i.e., larger and heavier leaves) of *B. pubescens* after manual clipping of opening apical buds and unfurling leaves.

Leaf quality in trembling aspen failed to improve despite having met each of the following two experimental conditions thought to be of importance in inducing amelioration in the foliage of other host plants (Bergström and Danell 1987, Haukioja et al. 1990, Senn and Haukioja 1994, Karban and Niiho 1995). The first of these conditions is that the most apically flushing buds were removed–presumably necessary in destroying branch-level apical dominance and thereby allowing previously suppressed basal buds to receive a greater share of the available nutrients (Haukioja et al. 1990, Karban and Niiho 1995). Secondly, bud-clipped and mined ramets did not show compensatory growth following the loss of approximately one-quarter of their expanding buds in the spring (Table 2.1). These conditions should increase the potential of allocating

more nutrients to each of the remaining juvenile shoots than would otherwise be possible in the unmanipulated trees (Bergström and Danell 1987).

One explanation for the lack of expected improvement in leaf quality of ramets after bud death relates to the amount of resources still available for the remaining buds under development. It is generally thought that plants have a limited amount of resources to support physiological processes (Herms and Mattson 1992). Given this, and the fact that I destroyed buds as leaves were beginning to emerge, too many resources may have been lost with the clipping or mining treatment to have had a significant ameliorating effect on the remaining juvenile shoots. This suggests that the phenolical window in which amelioration may be induced is extremely narrow. Some of the variable results found with bud removal treatments in mountain birch have also been attributed to this reason (Valanne and Valanne 1984, Senn and Haukioja 1994, Haukioja and Honkanen 1996). In support of this explanation is the birch study of Senn and Haukioja (1994), in which all bud removal treatments were implemented on the same day; late-flushing genets had improved leaf quality to a greater extent than early-flushing genets. Accordingly, when emergence of *M. disstria* larvae is prior to budbreak (especially on late flushing clones), and swollen rather than opening apical buds are mined (Ives and Wong 1988), perhaps only then do more resources per bud become available to ameliorate the quality of remaining leaves. Other early aspen feeding insect herbivores, such as large aspen tortrix (*Choristoneura conflictana* Walker), which prefer to colonize and feed in softening buds in the spring (Prentice 1955, Wickman 1963 and Parry, Volney,

and Currie 1997), may also have the ability to induce positive changes to their host's leaf quality.

The lack of induced amelioration found in this study may instead reflect the unique differences in requirements (i.e., damage cues) between aspen and mountain birch. Lastly, it may simply relate to the 'luck of the draw' in having inadvertently selected clones incapable of this response; aspen are well known for their high interclonal variability, in terms of response to herbivore damage (see interaction term in Table 2.2, Chapter 3). Clearly, a wider breadth of sampling of trembling aspen clones is required.

Results of my study are the first to suggest that particular clones of trembling aspen may actually become defended after the loss of expanding apical buds in the spring. Most notably, I found that trees from clone 1, that had been manually clipped in the current year (1998), had the most dramatic reductions in leaf quality; larvae performed significantly worse on these trees than on the controls of clone 1 (see Figs. 2.7 b, 2.9 b, 2.11 b and 2.12 b). However, larvae from clone 1 that were fed leaves from ramets whose buds were removed by insects in 1998, did not differ significantly in their performance from individuals fed from unclipped trees (Figs. 2.7 c to 2.12 c).

It was puzzling that 'natural' bud damage failed to induce similar defensive responses in clone 1, when other studies have found that insect feeding triggers stronger induced defenses than simulated damage (Haukioja and Neuvonen 1985, Hartley and Lawton 1987, 1991). Apparently, the natural and artificial treatments of clone 1, were qualitatively different from one another (Figs. 2.7 d,

2.11 d and 2.12 d), because quantitatively, they both destroyed approximately the same proportion of buds (see total bud/shoot mortality for 1998 - Table 2.1). These differences are most likely an effect of the rate and manner in which tissues were removed (Baldwin 1988, 1990), rather than an effect of the mesh bag used to confine larvae to particular buds. Recall that similar procedural studies have found bags to have negligible effects on the larval growth of particular insect herbivores (i.e., *E. autumnata* – Kaitaniemi et al. 1997; *M. disstria* - Parry et al. 1998). One might then speculate that it is the piece-meal progression of natural insect feeding by forest tent caterpillars that prevents the ramets from sensing and responding defensively [see Tallamy (1985), Dussourd and Eisner (1987), Sagers (1992) for examples of other insect defoliator species known to have adapted feeding habits that sabotage the defensive responses of their host plant].

If, however, the natural and artificial bud removal treatments varied strictly because of the bag effect, induced defenses in clone 1 could then be seen as rapid and short-term responses to bud removal (Haukioja and Neuvonen 1987): rapid because changes occurred in the same season as the damage, short-term because changes did not last beyond the current year of damage (see Figs. 2.7 a to 2.12 a for clone 1). Similar short-term induced defenses have previously been reported to occur in trembling aspen, but only after fully expanded leaves were artificially damaged (Clausen et al. 1989, Mattson and Palmer 1988).

Rapid, short-term induced defenses act to suppress the insect herbivore generation doing the damage and may therefore stabilize the population

(Haukioja and Neuvonen 1987). In my study, this is most likely to have occurred with caterpillars feeding on clone 1, given that individuals from the artificially clipped trees of 1998 took 1.4 days longer to complete development of the fourth stadium than individuals from control ramets (Fig. 2.7 b). This greatly protracted development of larvae has been found to increase the risk of mortality for insect defoliators as they are more likely to encounter predators and parasitoids (Schultz 1983, Parry et al. 1998).

The second method of assessing leaf quality used in my study, involved calculating average leaf size and testing whether this differed among 'treatments', 'clones', and 'clone by treatment interactions'. Leaf area did not differ significantly between treatments, in neither overall effects nor in clones separately (Table 2.3), yet larval performance measures did. From this, I conclude that leaf size was not a good indicator of induced changes in leaf quality of trembling aspen. This is in contrast with Clausen et al. (1991), who found leaf size of aspen to be an excellent predictor of leaf quality, measured by pupal masses of large aspen tortix (for further examples see Werner 1979, Haukioja 1980, Baltensweiler 1985).

Conversely, leaf area was an excellent predictor of overall leaf quality for the different aspen clones (Table 2.3), because the results mirrored those of the bioassays. Both leaf size and larval performance measures indicated that leaf quality was significantly poorer in clone 1 than in the other clones (Figs. 2.5 and 2.6). These results corroborate other findings of significant clonal variation in resistance to attack by forest tent caterpillars. Hwang and Lindroth (1997) found

that forest tent caterpillars had significantly different instar durations, growth and consumption rates when fed on 13 different aspen clones; their best clone completed development of the fourth stadium 2.4 days faster than those from their worst clone. This is comparable to my finding of a 2.1 day difference between clone 1 and 3 or 4 (Fig. 2.5 a). Robison and Raffa (1994) also determined that the feeding preference of second and fourth instar forest tent caterpillars differed significantly on various clones of hybrid poplar.

Interclonal variation in aspen's defensive chemistry was likely responsible for the variable results observed among the aspen clones of my study. This is furthermore a genetically based characteristic and not merely the result of site variations, since two of the experimental clones (i.e., clones 1 and 2), situated less than 5 meters apart, showed significant differences in leaf quality (see Figs. 2.1 and 2.6 a).

I have shown in this study that real or simulated larval feeding damage to young unfurling leaves in the spring, did not appear to induce positive changes in the leaf quality of trembling aspen clones. Instead, leaf quality for forest tent caterpillars remained unchanged or declined significantly when fed from clipped ramets of one clone in particular. Currently, the absence of specific data about induced amelioration does not warrant the conclusion that it is unimportant in regulating insect population densities. If, for instance, a number of clones in a forest do increase their susceptibility to *M. disstria* attack following moderate levels of early feeding damage to the opening buds, then densities of the

herbivore may rise to epidemic levels. The outbreak could then spread from this epicenter into the remaining forest (Haukioja 1990).

2.6 LITERATURE CITED

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Table 2.1. Percent mortality, production and overall loss or gain of buds/shoots in a growing season for variously treated and untreated trembling aspen ramets of different clones (mean ± SE).

1997	Bud Removal Treatments		Mann-Whitney Test
	25% Artificial [a]	Control [b]	(2-tailed)
Background Bud/Shoot Mortality (%)	4.88 ± 1.13 (n = 8)	4.00 ± 1.84 (n = 7)	U' = 34.5, NS
Total Bud/Shoot Mortality (%) [d]	28.50 ± 1.25 (n =8)	4.00 ± 1.84 (n = 7)	U' = 56.0, P < 0.05
New Shoots Produced (%) [e]	2.00 ± 1.32 (n = 8)	0.29 ± 0.29 (n = 7)	U' = 38.0, NS
Difference btw Potential and Actual Shoot # (%) [f]	-26.75 ± 1.77 (n = 8)	-3.71 ± 1.76 (n = 7)	U' = 56.0, P < 0.05

1998	Bud Removal Treatments		Mann-Whitney Test
	25% Natural [c]	25% Artificial [a]	(2-tailed)
Background Bud/Shoot Mortality (%)	5.17 ± 1.22 (n = 6)	4.67 ± 2.06 (n = 6)	U' = 21.0, NS
Total Bud/Shoot Mortality (%) [d]	27.33 ± 1.43 (n = 6)	28.00 ± 1.53 (n = 6)	U' = 20.5, NS
New Shoots Produced (%) [e]	2.50 ± 0.96 (n = 6)	3.83 ± 1.33 (n = 6)	U' = 23.0, NS
Difference btw Potential and Actual Shoot # (%) [f]	-25.17 ± 1.47 (n = 6)	-25.33 ± 1.93 (n = 6)	U' = 18.5, NS

[a] Opening buds removed by manually clipping with pruning shears

[b] Ramets that had no buds removed by the experimenter (i.e. Control)

[c] Opening buds mined by first instar forest tent caterpillar

[d] Total percent of buds/shoots destroyed by the treatments and natural causes

[e] New shoots arising from the activation of latent buds (see Fig. 2.3)

[f] Difference between # of buds (potential shoots) in spring and # of shoots in late summer of the same year

Only Mann-Whitney statistics followed by *P* values indicate that comparisons were significantly different.

Table 2.2. ANOVA tables for various performance measures of fourth instar forest tent caterpillars.

		Instar Duration			RGR			RCR		
2-Way ANOVA	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Model										
Clone	2	171.91	193.27	< 0.001	34.11	271.12	< 0.001	4109.47	340.12	< 0.001
Treatment	3	3.27	3.67	0.012	0.10	0.82	0.485	201.56	16.68	< 0.001
Clone x Treatment	6	4.07	4.57	< 0.001	0.62	4.95	< 0.001	66.28	5.49	< 0.001
Error	390	0.89			0.13			12.08		

		AD			ECD			ECI		
2-Way ANOVA	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Model										
Clone	2	3235.60	57.96	< 0.001	1033.84	35.67	< 0.001	699.52	85.31	< 0.001
Treatment	3	1041.38	18.65	< 0.001	296.43	10.23	< 0.001	62.21	7.59	< 0.001
Clone x Treatment	6	197.57	3.54	0.002	119.15	4.11	< 0.001	39.33	4.80	< 0.001
Error	389	55.82			28.98			8.20		

Table 2.3. ANOVA table for leaf area.

1997		**Leaf Area**		
2-Way ANOVA	df	MS	*F*	*P*
Model				
Clone	3	19.28	13.64	0.003
Treatment	1	5.65	4.00	0.086
Clone x Treatment	3	1.38	0.98	0.456
Error	7	1.41		

1998		**Leaf Area**		
2-Way ANOVA	df	MS	*F*	*P*
Model				
Clone	2	29.35	20.73	< 0.001
Treatment	3	1.41	1.00	0.428
Clone x Treatment	6	2.32	1.64	0.219
Error	12	1.42		

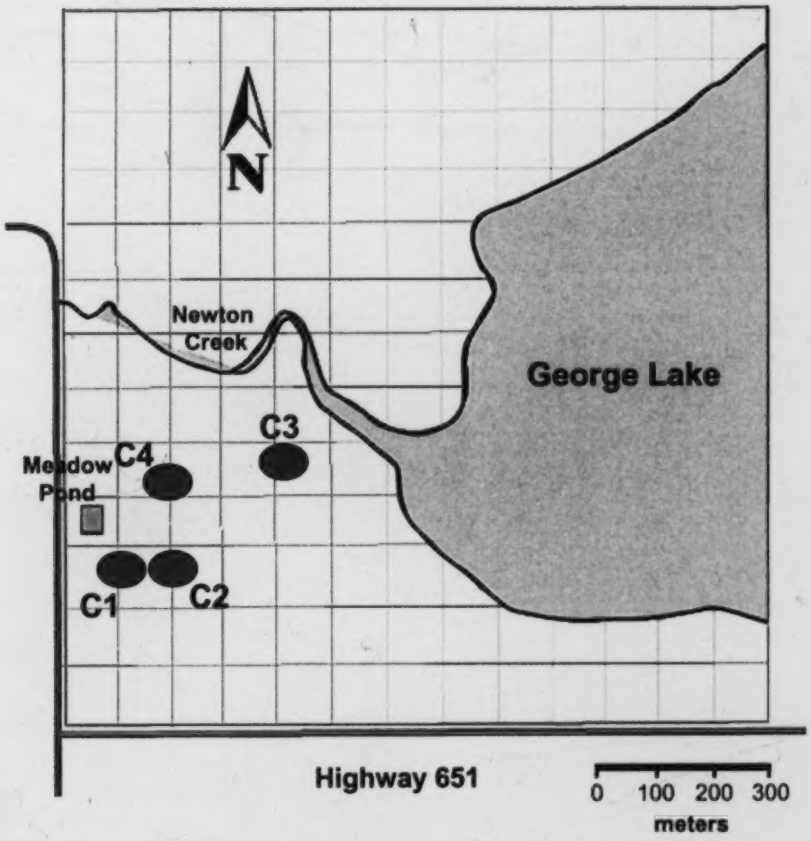

Fig. 2.1. Study location and spacial arrangement of trembling aspen clones.

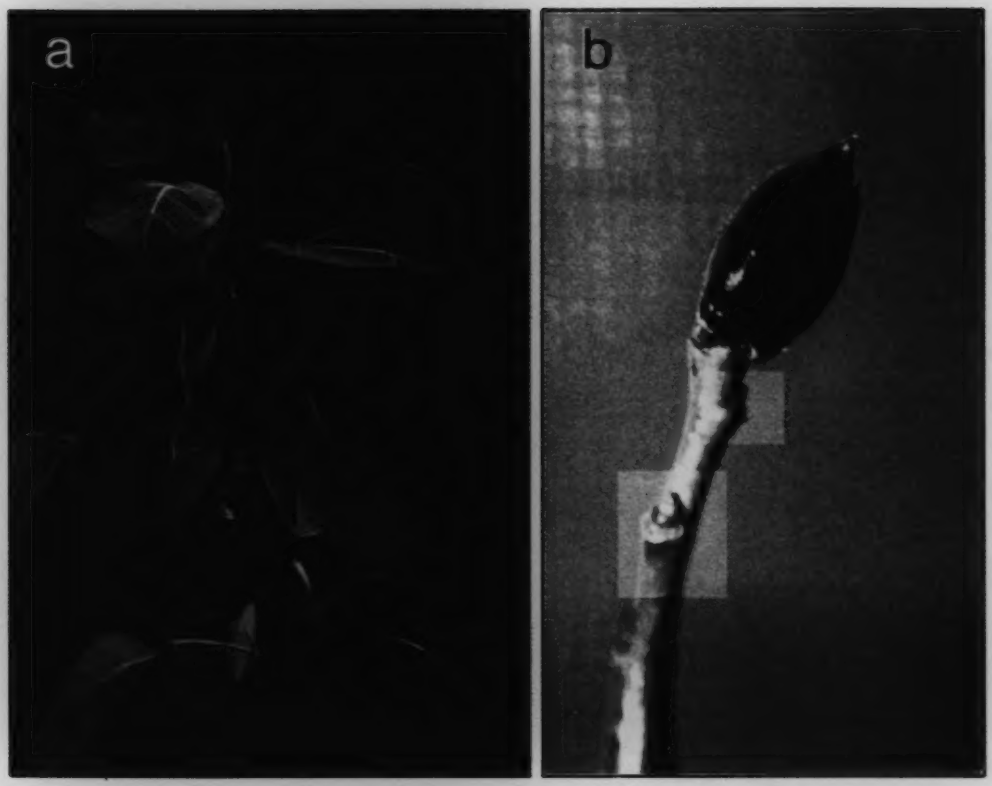

Fig. 2.2. (a) Two forest tent caterpillar egg bands deposited near the apex of a trembling aspen branch. (b) Examples of latent buds of *P. tremuloides* (source: Canadian Forest Service, printed with permission).

Fig 2.3. A latent bud activated to develop into a new shoot after the active terminal bud of this branch was destroyed.

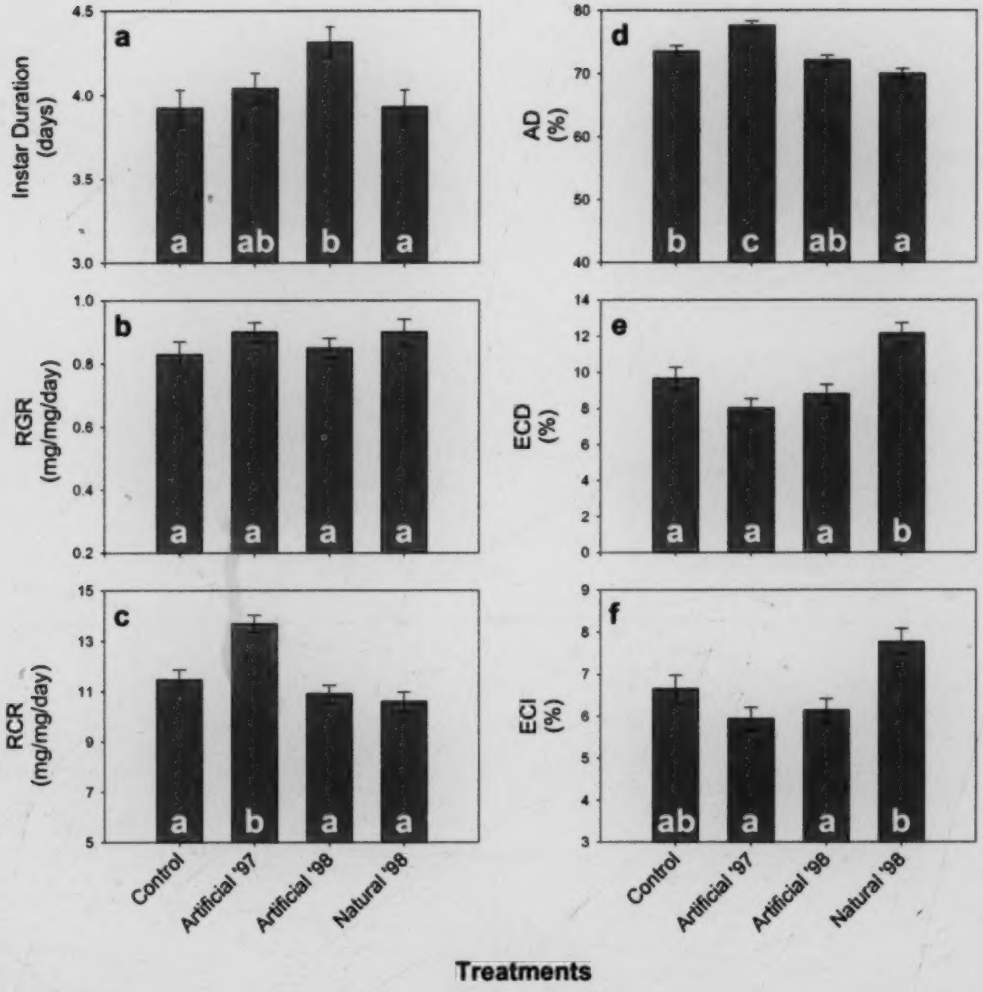

Fig. 2.4. Least square mean (± SE) of instar duration (a), RGR (b), RCR (c), AD (d), ECD (e) and ECI (f) for fourth instar (L4) forest tent caterpillars feeding on control ramets, artificially clipped ramets from 1997 or 1998, and naturally mined ramets from 1998. Only pre-planned pairwise comparisons ($P = 0.05$) with sequential Bonferroni adjustments were made. Bars with the same letter indicate no significant difference.

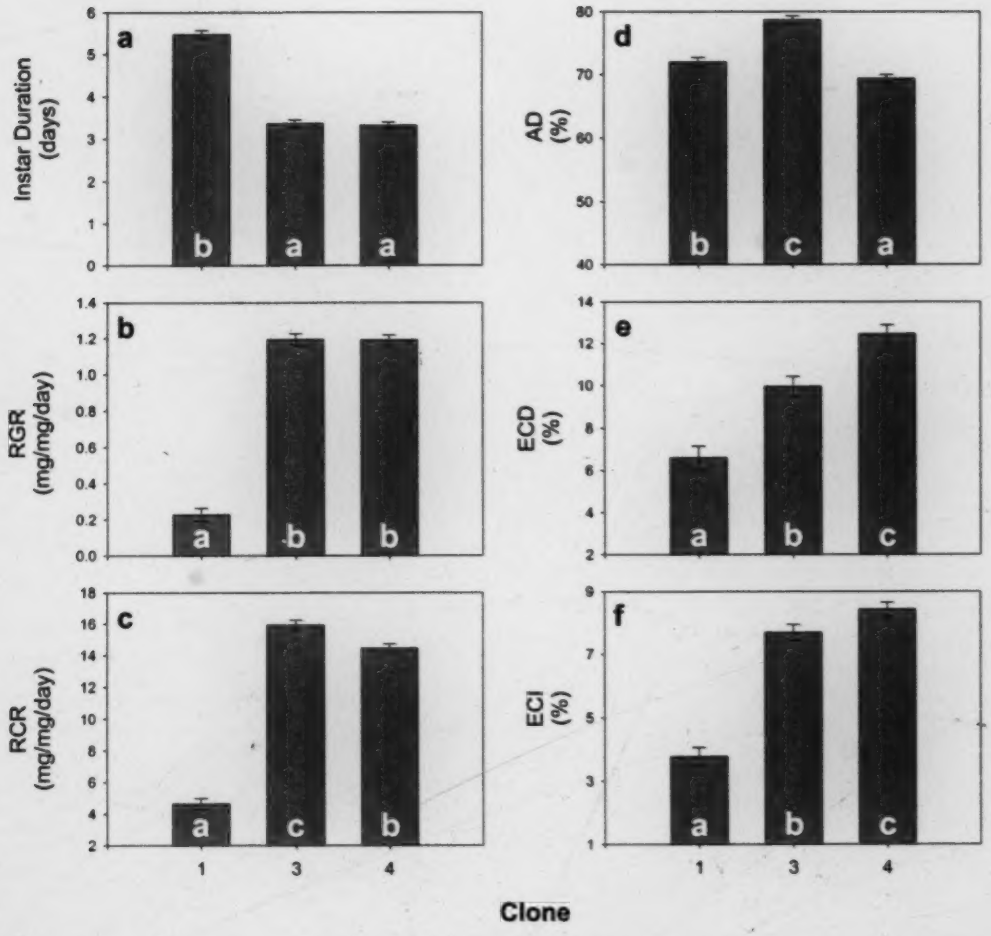

Fig. 2.5. Least square mean (± SE) of instar duration (a), RGR (b), RCR (c), AD (d), ECD (e) and ECI (f) for fourth instar (L4) forest tent caterpillars feeding on three clones of trembling aspen. Only pre-planned pairwise comparisons ($P = 0.05$) with sequential Bonferroni adjustments were made. Bars with the same letter indicate no significant difference.

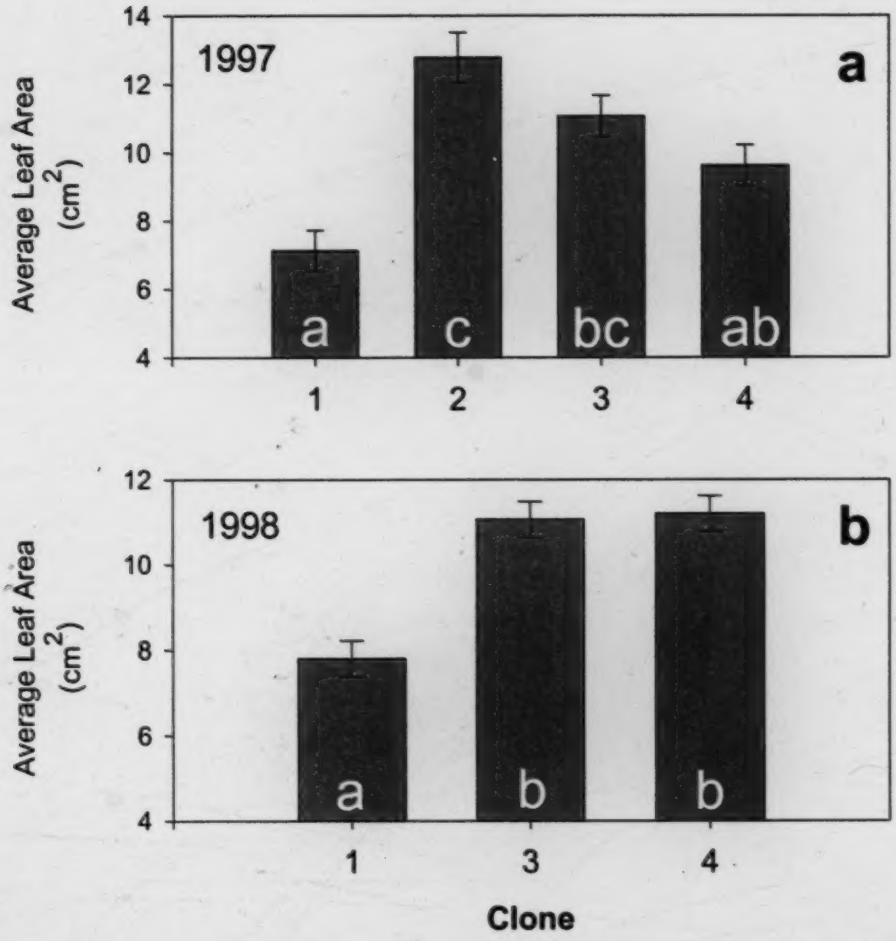

Fig. 2.6. Least square means (± SE) of average leaf area (a) for four clones of trembling aspen in 1997 and (b) for three of the same clones in 1998. Only pre-planned pairwise comparisons ($P = 0.05$) with sequential Bonferroni adjustments were made. Bars with the same letter indicate no significant difference.

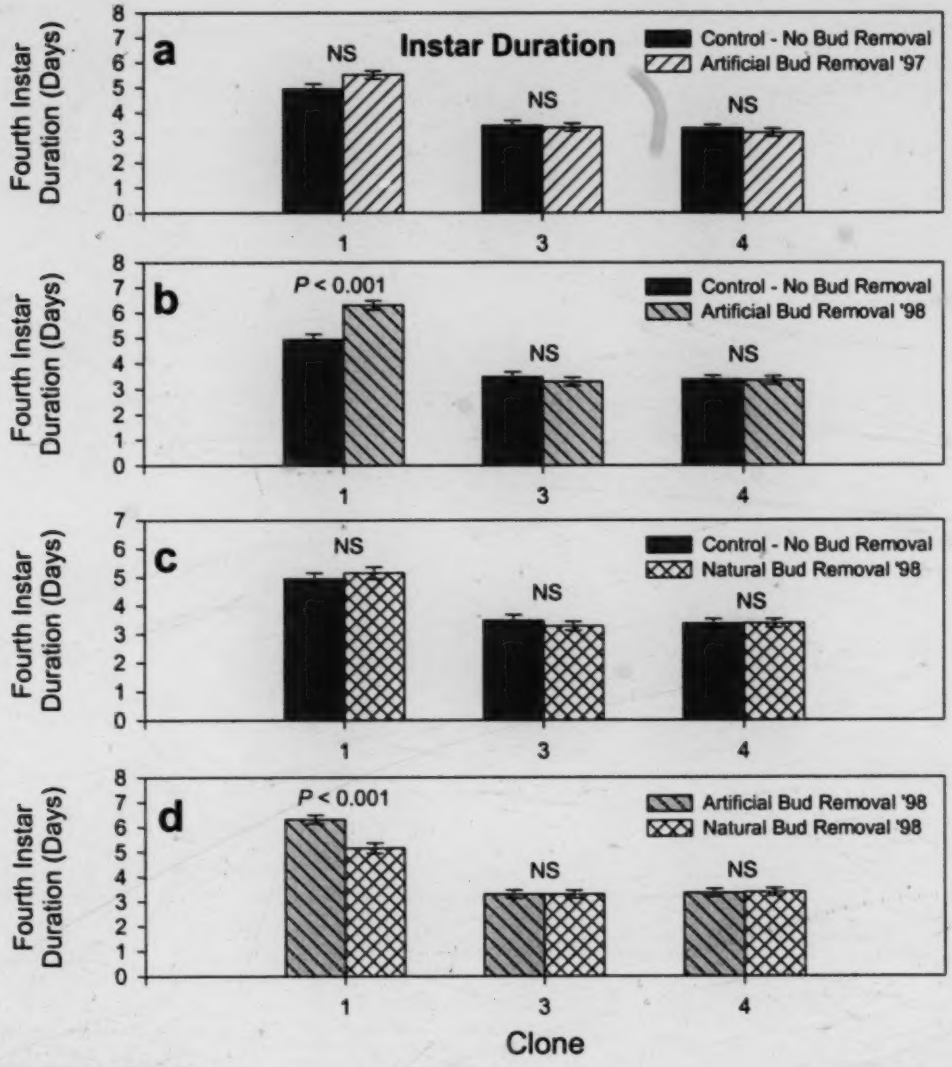

Fig. 2.7. Comparisons of least square means (± SE) of instar duration for fourth instar tent caterpillars feeding on (a) ramets with buds artificially removed in 1997 (striped bars) and control ramets (solid coloured bars), (b) ramets with buds artificially removed in 1998 (striped bars) and control ramets, (c) ramets with buds naturally mined by larvae in 1998 (cross hatched bars) and control ramets, finally, (d) ramets with buds removed artificially in 1998 and those naturally mined in 1998 from different aspen clones. Comparisons with *P* values indicate significant differences following sequential Bonferroni adjustments, however, those with NS indicate no significant differences.

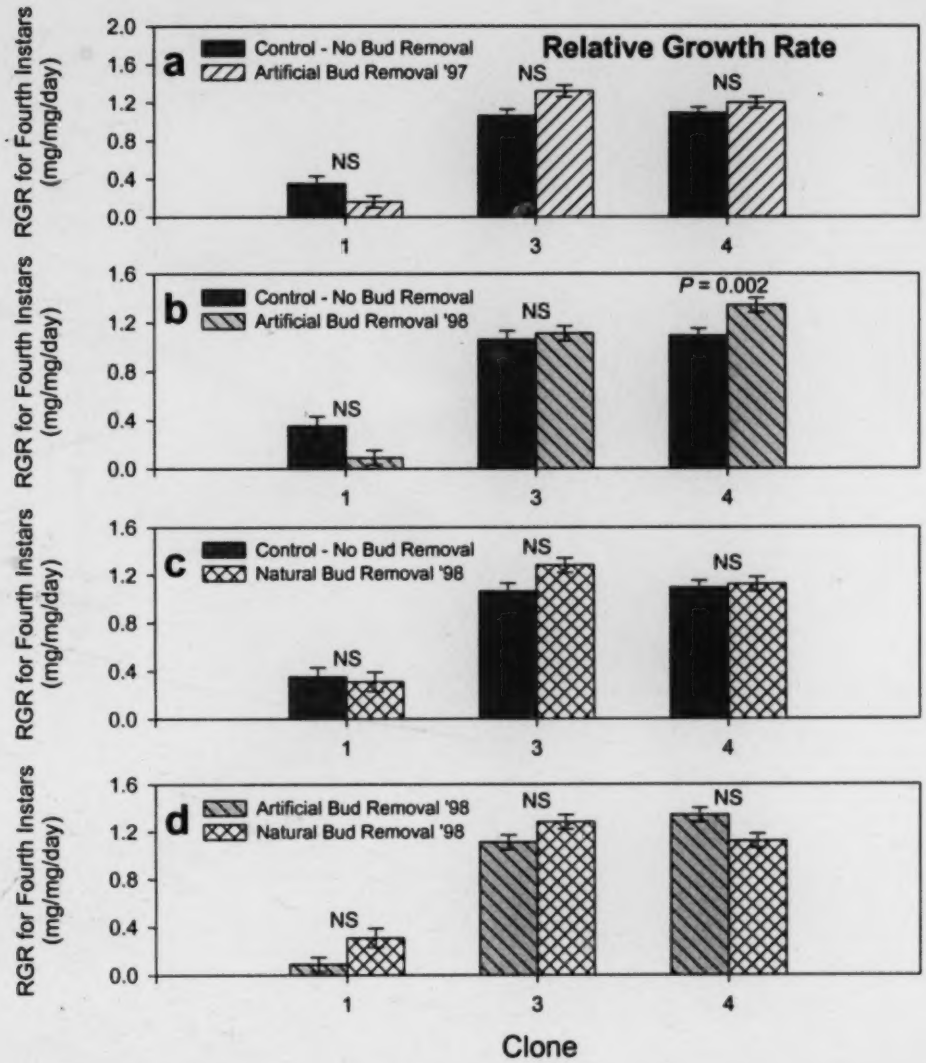

Fig. 2.8. Comparisons of least square means (± SE) of Relative Growth Rate (RGR) of fourth instar tent caterpillars feeding on (a) ramets with buds artificially removed in 1997 (striped bars) and control ramets (solid coloured bars), (b) ramets with buds artificially removed in 1998 (striped bars) and control ramets, (c) ramets with buds naturally mined by larvae in 1998 (cross hatched bars) and control ramets, finally, (d) ramets with buds removed artificially in 1998 and those naturally mined in 1998 from different aspen clones. Comparisons with *P* values indicate significant differences following sequential Bonferroni adjustments, however, those with NS indicate no significant differences.

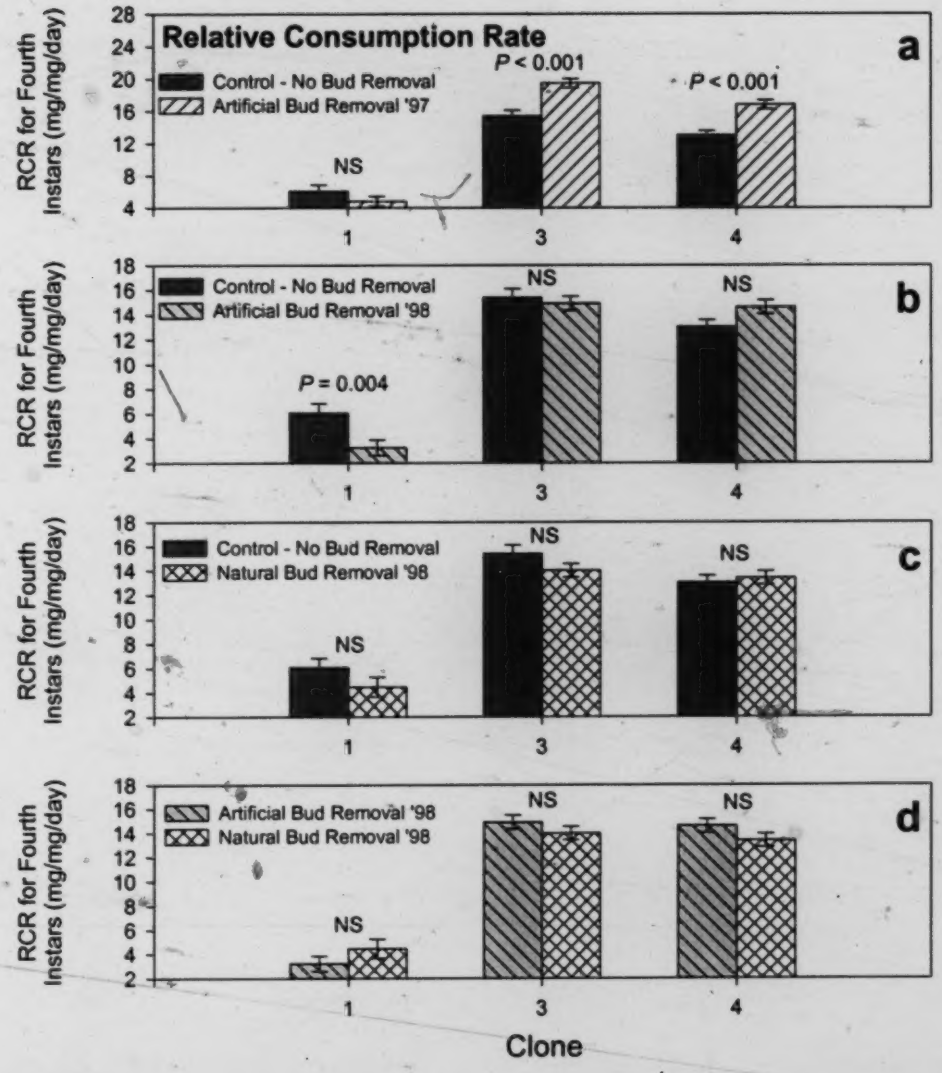

Fig. 2.9. Comparisons of least square means (± SE) of Relative Consumption Rate (RCR) of fourth instar tent caterpillars feeding on (a) ramets with buds artificially removed in 1997 (striped bars) and control ramets (solid coloured bars), (b) ramets with buds artificially removed in 1998 (striped bars) and control ramets, (c) ramets with buds naturally mined by larvae in 1998 (cross hatched bars) and control ramets, finally, (d) ramets with buds removed artificially in 1998 and those naturally mined in 1998 from different aspen clones. Comparisons with *P* values indicate significant differences following sequential Bonferroni adjustments, however, those with NS indicate no significant differences.

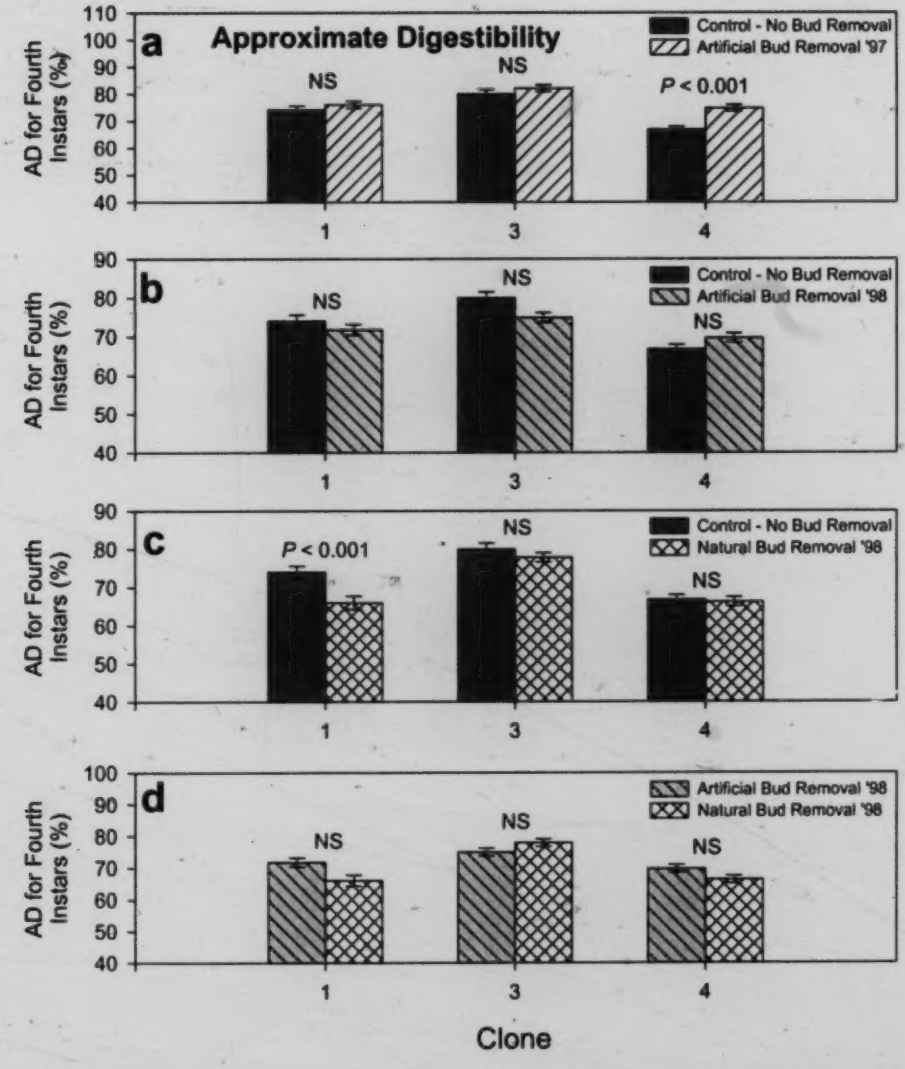

Fig. 2.10. Comparisons of least square means (± SE) of Approximate Digestibility (AD) of fourth instar tent caterpillars feeding on (a) ramets with buds artificially removed in 1997 (striped bars) and control ramets (solid coloured bars), (b) ramets with buds artificially removed in 1998 (striped bars) and control ramets, (c) ramets with buds naturally mined by larvae in 1998 (cross hatched bars) and control ramets, finally, (d) ramets with buds removed artificially in 1998 and those naturally mined in 1998 from different aspen clones. Comparisons with *P* values indicate significant differences following sequential Bonferroni adjustments, however, those with NS indicate no significant differences.

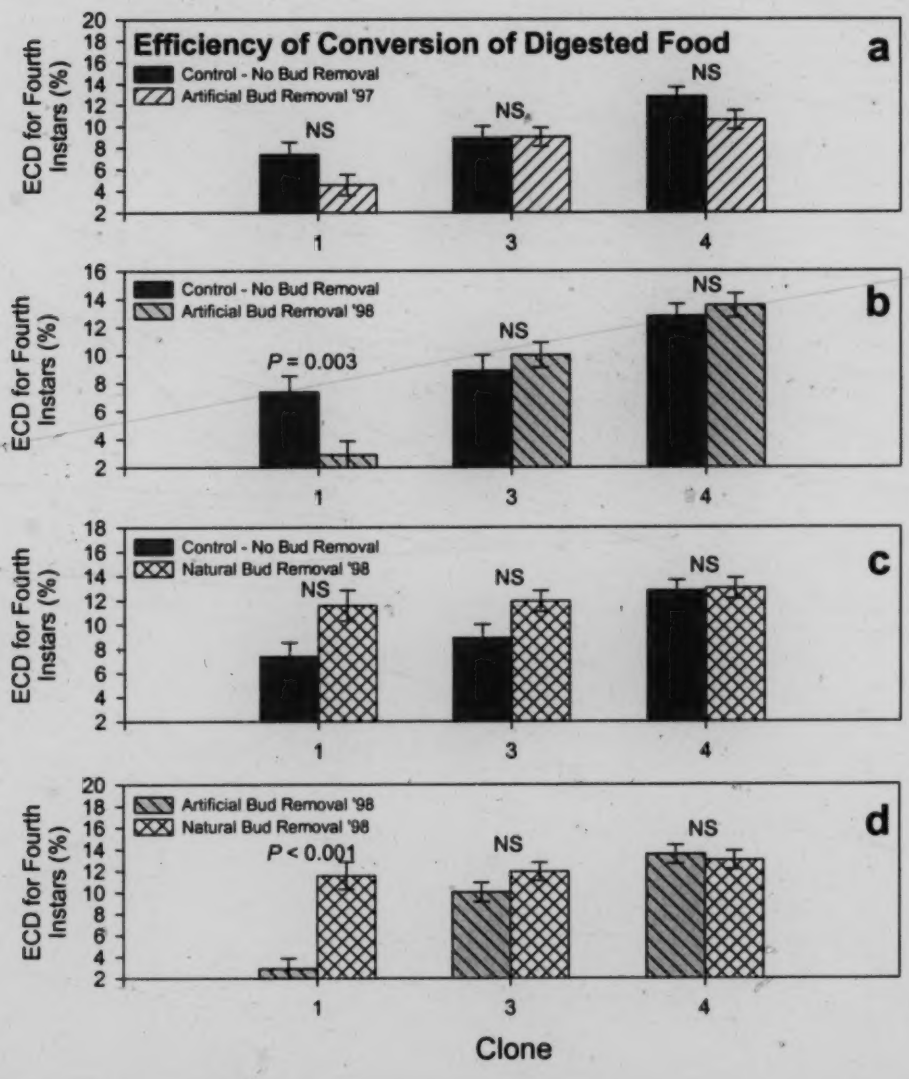

Fig. 2.11. Comparisons of least square means (± SE) of Efficiency of Conversion of Digested food (ECD) of fourth instar tent caterpillars feeding on (a) ramets with buds artificially removed in 1997 (striped bars) and control ramets (solid coloured bars), (b) ramets with buds artificially removed in 1998 (striped bars) and control ramets, (c) ramets with buds naturally mined by larvae in 1998 (cross hatched bars) and control ramets, finally, (d) ramets with buds removed artificially in 1998 and those naturally mined in 1998 from different aspen clones. Comparisons with *P* values indicate significant differences following sequential Bonferroni adjustments, however, those with NS indicate no significant differences.

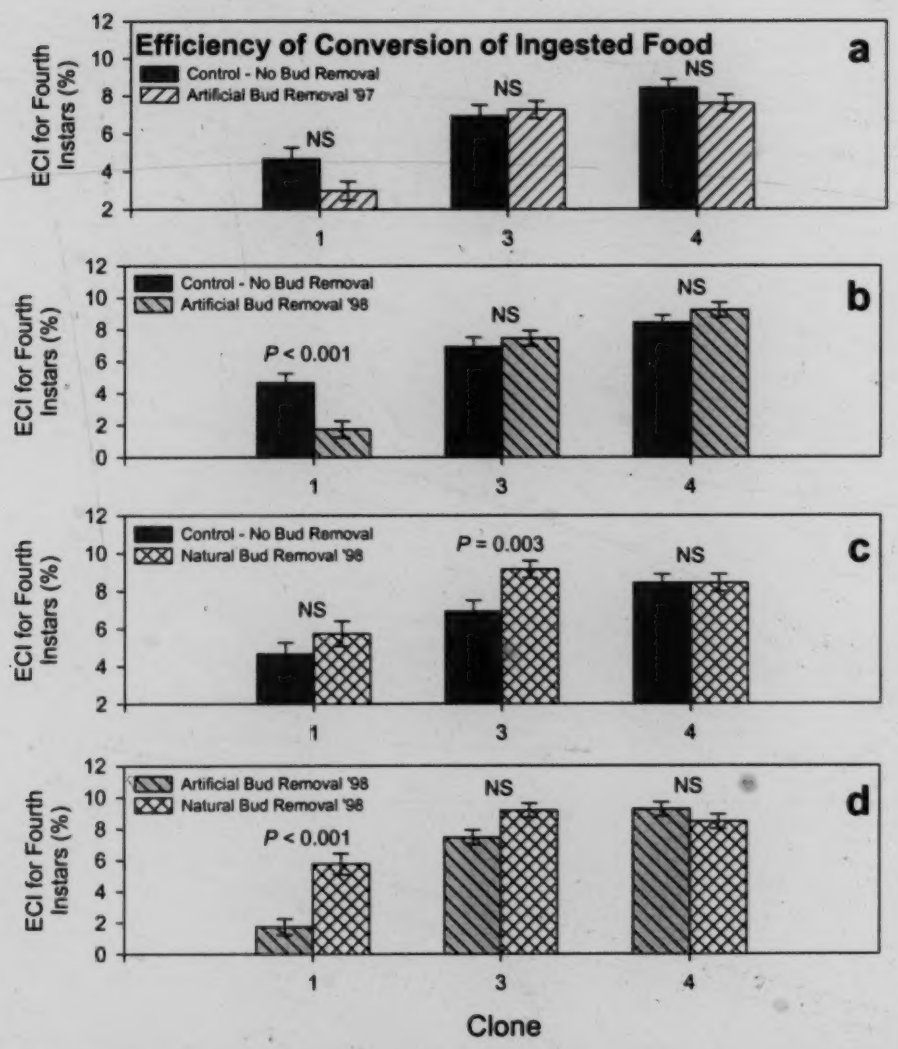

Fig. 2.12. Comparisons of least square means (± SE) of Efficiency of Conversion of Ingested food (ECI) of fourth instar tent caterpillars feeding on (a) ramets with buds artificially removed in 1997 (striped bars) and control ramets (solid coloured bars), (b) ramets with buds artificially removed in 1998 (striped bars) and control ramets, (c) ramets with buds naturally mined by larvae in 1998 (cross hatched bars) and control ramets, finally, (d) ramets with buds removed artificially in 1998 and those naturally mined in 1998 from different aspen clones. Comparisons with *P* values indicate significant differences following sequential Bonferroni adjustments, however, those with NS indicate no significant differences.

Chapter 3

Long-term induced defenses in four clones of trembling aspen

3.1 SYNOPSIS

In this chapter, I examine the effects that natural and simulated defoliation of trembling aspen (*Populus tremuloides* Michaux) had on the growth and feeding efficiencies of forest tent caterpillar (*Malacosoma disstria* Hübner) larvae. I also tested whether long-term induced defenses were localized or systemic plant responses. Ramets from four aspen clones were 75% defoliated in mid June 1997 by forest tent caterpillars or manually clipped using scissors. In 1998, bioassays were conducted with first and fourth instar larvae, which were fed in the field and laboratory, respectively. Larvae reared from leaves of naturally defoliated trees gained less biomass, and had lower RGRs and ECIs than larvae fed from the controls. This indicates that defenses were induced in aspen from one year to the next. Insect damage appeared to elicit stronger induced defenses than mechanical wounding. Plant defenses were systemically induced, thus conferring protection for the entire ramet. Leaf quality varied dramatically between clones in the absence of defoliation. Significant interclonal variation in response to defoliation treatments was also found. The potential effects of long-term induced defenses on the population dynamics of forest tent caterpillars are briefly discussed.

3.2 INTRODUCTION

Much of the research on forest tent caterpillars (*Malacosoma disstria* Hübner) has focused on possible causes of decline and eventual collapse in cyclic population outbreaks. Both density-independent and density-dependent factors have been proposed. Documented examples of density-independent factors include abnormally low spring (Blais et al. 1955) and winter temperatures (Witter et al. 1975). Density-dependent factors, on the other hand, include disease (Anonymous 1986), pupal parasitism (Hodson 1941, Witter et al. 1972), and starvation due to extreme defoliation of the host plant (Smith and Goyer 1986). However, none of these factors, offer a sufficient general explanation of why populations crash. For instance, inclement weather (Daniel 1990) and disease (Wellington 1960) are not always associated with declining populations. Regulatory roles of predators and parasitoids must also be interpreted cautiously, due to their variability in space and time (Parry, Spence and Volney 1997). Local correlation may easily be confused with causation in these interactions (Price 1987). Furthermore, in some cases outbreaking populations decline long before foliage quantity becomes limiting, thus excluding starvation as a general factor (Myers 1993).

Another density-dependent factor, strongly associated with population crashes in other cyclical lepidopterans, is deterioration of host quality as a result of insect feeding (Baltensweiler et al. 1977, Benz 1974, 1977; Haukioja and Hakala 1975; Haukioja 1990; Haukioja et al. 1990). These changes in foliage

quality that result from injury are known as "induced responses" (Karban and Myers 1989). Perhaps the most frequently cited induced response of deciduous trees is an increase in concentrations of secondary plant metabolites, namely phenolic compounds (Baldwin and Schultz 1983, Bryant et al. 1991, Clausen et al. 1989, 1991, Mattson and Palmer 1988). However, other studies have also found increases in leaf fiber (Benz 1974), and/or decreases in nitrogen (Benz 1974, Bryant et al. 1991) or water (Hartley and Lawton 1987) following true and simulated insect herbivory.

Herbivores feeding on plants with induced response may be adversely affected by having reduced fecundity (Raupp and Denno 1984, Rhoades 1983a), prolonged development times (Haukioja and Neuvonen 1985), reduced growth (Haukioja and Neuvonen 1985) and increased mortality (Benz 1974). Changes in foliage that elicit these responses are termed "induced defenses" (Wagner 1988). Induced defenses in some plants may persist a number of years, perhaps even after the insect population outbreak has returned to endemic levels (Haukioja and Neuvonen 1987). As a result, the average fitness in subsequent generations of herbivores may be adversely affected, thereby preventing the population from returning to outbreak levels for several years. These defenses can introduce the time lags necessary to obtain cyclic population dynamics. Numerous terms have been used to describe this phenomenon; I refer to it in this chapter as "long-term induced defenses".

There are no studies of the potentially important but poorly understood effects of long-term induced defenses on the growth and feeding efficiency of

forest tent caterpillar larvae. The main objective of my study, therefore, is to test whether long-term induced defenses occur a year following significant defoliation in trembling aspen (*Populus tremuloides* Michaux), this insect's primary host. The clonal origin of foliage used in the bioassays was also examined as a source of variation in forest tent caterpillar responses, because aspen clones often vary in their resistance to attack by herbivores (Hemming and Lindroth 1995, Hwang and Lindroth 1997, Robison and Raffa 1994). It was further tested whether 'natural' insect defoliation induces stronger defensive responses in aspen, than simulated or 'artificial' defoliation. Finally, to determine if induced defenses are local or systemic tree responses, larvae were fed leaves from previously defoliated or non-defoliated branches of the same tree.

3.3 MATERIALS AND METHODS

3.3.1 Site Description and Clone Selection

The study was conducted between 1997 and 1998, at the George Lake Field Station (114°06'W, 53°57'N), approximately 75 km northwest of Edmonton, in central Alberta, Canada. The forest was dominated by mature to over-mature trembling aspen, which had experienced moderate to severe defoliation by forest tent caterpillar throughout most of the 1980s (Parry, Spence and Volney 1997). By 1996, new aspen saplings representing various clones had become well established in the gaps of the more decadent stands.

Ramets of four different trembling aspen clones were identified by observing leaf flush times in the spring and leaf shape once the blade was fully expanded (criteria outlined by Barnes 1969, Steneker and Wall 1970). Clones selected for study had to have at least 8 ramets meeting the following requirements: 1) suitable size (height = 4 – 6 m; diameter at breast height = 20 – 30 mm); 2) a shape free of major crooks in the main stem; 3) be free of disease symptoms and hare/deer browsing damage; 4) growing in the open or in partial shade. Clones were also selected in relatively close proximity to one another, so that site variations could be minimized (Fig. 3.1). Four suitable clones were found and mapped in the upland aspen forest (see Niemelä et al. 1992 for detailed description of the forest type). Tree ring counts, made at the end of the experiment, indicated that the ramets were 11-13 years old. Thus the trees used in this study may have been previously defoliated as very young shoots because the last forest tent caterpillar outbreak at George Lake did not collapse until 1989.

3.3.2 Defoliation treatments

Significant long-term induced defenses, generally require severe defoliation (Haukioja et al. 1983), however, preliminary work done in 1996, showed that 100% defoliation greatly increased the probability of mortality in young trees. For this reason I removed only the top 75% of the leaves in this experiment. Leaves were completely removed in two ways, either by fifth instar forest tent caterpillar larvae (natural defoliation), which were enclosed on

branches with bags, or by clipping with scissors (artificial defoliation), which did not require the use of bags. Two kinds of undefoliated controls were also used, one with bags and one without.

These four treatments were randomly assigned to ramets and replicated twice (4 x 2 = 8 ramets) within each clone. Prior to defoliation, the number of leaves and location of each shoot on the ramet was recorded, so that the number of leaves required to achieve 75% defoliation could be determined.

Treatments were applied in mid June 1997, at the time when fifth instar forest tent caterpillars normally do the majority of their feeding in central Alberta. In the artificial defoliation treatment, standard household scissors were used to remove the entire blade, so that only the petiole remained. Cut leaf blades were collected and taken from the site. Two ramets from each clone were left completely undisturbed; they served as controls for the artificial defoliation treatment.

The natural defoliation treatment was administered by enclosing fifth instar forest tent caterpillar larvae on designated branches of ramets with fine mesh pollination bags (Fibe-Air, Kleen-Test Products, Brown Deer, WI). Larvae used for this treatment were taken from a natural outbreak near Donnelly, Alberta. The controls for this treatment were ramets, whose branches were similarly enclosed with bags but without caterpillars.

Whereas clipping of the artificially defoliated ramets took a few hours, the insect feeding on the naturally defoliated ramets took 3 to 7 days. Bags on control treatments remained on the ramets for the same amount of time that it

took caterpillars to eat most of the leaves (i.e., ~7 days). The artificial defoliation treatment in clone 1 and 3 removed more leaf tissue than the natural defoliation treatment because fifth instar larvae did not always consume the entire blade of the leaves. Specifically, in clone 1, larvae removed only 57.16 ± 1.08% of the leaves, while the manual clipping removed 75%. Differences between these treatments were not quantified for clone 3, but it appeared that larvae consumed 50% of the foliage.

3.3.3 Assessment of leaf quality

In the year following defoliation (1998), leaf quality was indirectly assessed through bioassays of first and fourth instar forest tent caterpillars. First instars were used because they are often more strongly affected by foliar changes than late larval instars (Scriber and Slansky 1981). However, penultimate stage larvae are technically easier to work with, when standard nutritional indices of larval performance are calculated (Scriber and Slansky 1981). Much more of the total consumption and growth of larvae also occurs during this later stage. Therefore, fourth instar performance values tend to be representative of those calculated for all larval stages (Scriber and Slansky 1981).

The larvae used in the present study were obtained from egg bands collected from a young outbreak near Flin Flon, Manitoba. They were kept over-winter in large brown paper bags at -10° C until April 1998. Only large, regular shaped egg bands with complete spumuline coverings were selected to produce

insects for the bioassays. Egg bands were scraped to remove the spumaline and surface sterilized according to the procedure outlined by Parry, Spence and Volney (1997) to prevent infection by nuclear polyhedrosis virus (NPV). Egg masses were then stored in a refrigerator at 4° C, until needed.

First instar larval feeding trials

The phenological development of trembling aspen buds was closely monitored at George Lake, beginning in late April 1998. Bud developmental stages were classified daily using the technique of Parry, Volney and Currie (1997). When most of the buds in one clone were in condition class 3 (i.e., green colouration visible along edges of bud scales, buds becoming soft), 10 sterilized egg bands were placed in a 25° C incubator and allowed to hatch. Within 2 - 3 days, larvae were sorted into groups of 15 individuals, randomly chosen from different egg bands to control for any differences among the progeny of females. Larvae were then held at 4° C in microfuge tubes (1.5 mL), pending their relocation to trees.

Larvae were placed on each clone at the time of budbreak, when the majority of buds on the ramets were in the 6th condition class (i.e., bud scales shed, leaves elongating and unrolling) (Parry, Volney and Currie 1997). Leaf-out varied among clones; clone 1 flushed first, on May 3, followed by clone 2, three days later. Budbreak occurred on May 9th and 13th for clones 4 and 3, respectively. Synchronizing the insect and plant phenology was important

because caterpillars that feed out of synchrony with their host, experience prolonged development times and reduced survival (Parry et al. 1998).

To test whether long-term induced defenses were local or systemic responses, two groups of caterpillars were placed in separate bags at the top, middle and bottom of the canopy (6 groups/ramet). Larvae that had been placed at the top and middle of the canopy, fed on branches that had previously been defoliated. Conversely, larvae placed at the bottom, fed on branches that had not previously been defoliated. No-see-um netting (Totem Outfitters, Edmonton, AB) was used to construct the 25cm x 15cm bags. Microfuge tubes, containing larvae, and bags themselves were fastened to the branch with 'twist tie' wires. After the bag was secured, the microfuge tube was opened and tapped to allow larvae to fall out onto the bag or the unfurling leaves. The insects were thus confined on the tree for two weeks.

Percent mortality was calculated based on the number of larvae missing or dead from each bag after two weeks. Caterpillars were then frozen, dried (at 50° C for 48 hours) and weighed on a Cahn 26 Automatic Electrobalance (accuracy ± 1.0 μg) to determine the change in biomass averaged for each group [average final mass per larva (i.e. group mass ÷ number of remaining caterpillars) – average initial mass per larva]. All first instar larvae were assigned the same initial mass of 0.064 mg (± 0.00038 mg), because it was not feasible to establish the average mass per larva for each group of caterpillars used in the experiment.

Fourth instar larval feeding trials

The remaining egg bands were allowed to hatch at room temperature. Larvae from different egg bands were combined and reared on the same field-collected foliage until they reached the end of the third stadium. Newly molted fourth instars were weighed and used for feeding trials that began in late May, 1998. Caterpillars were reared individually in 150-ml wax paper cups (Lily Cups Inc., Scarborough, Ont.) at a constant temperature of 25° C, under a 16 : 8 (L : D) photoperiod. Fourth instars were randomly assigned foliage from one of the experimental ramets for the duration of the feeding trial. A total of 20 larvae were reared on each ramet (20 x 8 x 4 = 640). The leaves, they received, came from the top and bottom of the canopy and were first weighed to obtain wet leaf masses for future calculations. The petioles of the leaves were wrapped in cotton and placed in small glass vials of water to keep them fresh. Foliage was replaced every 2 days until larvae completed the fourth stadium, at which point they were frozen. The checking interval for new fifth instars was every 2 hours except between 12 midnight and 6 am. Feeding trials were terminated after 7.5 days because foliage on some experimental trees had been exhausted. Caterpillars that failed to molt within that time were frozen and still used for calculation of nutritional indices. Development time for each larva was calculated in days.

Caterpillars were later thawed and sexed (Stehr and Cook 1968, p. 217) to partition data between males and females. Sexed larvae, frass and uneaten

leaves were dried (at 50° C for 48 hrs) and weighed. Standard nutritional indices were calculated for each larva, including relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested (ECD) and ingested (ECI) food (Scriber and Slansky 1981, Waldbauer 1968) (see Appendix 1 for general calculations of nutritional indices). The initial dry mass of leaves were estimated from regression analysis of the wet to dry mass ratio of variably sized leaves from the four clones ($y = 0.33 x + 8.55$, $n=315$, $P < 0.001$, $r^2=0.96$). One hundred newly molted fourth instar larvae, that were sacrificed before the feeding trails began, were also used to develop regression estimators of initial dry mass based on the live mass of individuals ($y = 0.134 x - 0.117$, $n = 100$, $P < 0.001$, $r^2 = 0.93$).

3.3.4 Statistical Analysis

A three-way analysis of variance (ANOVA) with interactions was used for the first instar bioassays (PROC GLM procedure in SAS, SAS Institute1996). This analysis tested the effects of clonal origin of foliage, defoliation treatments on the ramet and larval feeding position in the canopy on percent larval mortality and biomass gain. For each of the significant main effects and interactions, pre-planned pairwise *t*-tests (P=0.05) (LSMEANS statement, PDIFF option) were performed (SAS Institute1996), with adjustments for experimentwise Type I errors using a sequential Bonferroni test (Rice 1989), to ascertain significance of differences. Comparisons of a priori interest were larval performance 1) among the four clones; 2) among the four treatments; 3) between artificially defoliated

and non-defoliated ramets without bags by clone; 4) between naturally defoliated and non-defoliated ramets with bags by clone; 5) between artificially and naturally defoliated ramets by clone; 6) between non-defoliated ramets with and without bags by clone.

The fourth instar bioassay was also analyzed using the three-way ANOVA procedure described above to determine the effects of clone, defoliation treatment and sex on instar duration, RGR, RCR, AD, ECD and ECI. The same pre-planned pairwise comparisons as outlined above were made among main effect and interaction least-square means.

3.4 RESULTS

The recent defoliation history of aspen ramets affected forest tent caterpillar performance. Analysis of first instar feeding trials revealed that larval weight gain varied significantly with defoliation treatment ($F_{3,130} = 6.63$; $P < 0.001$, Table 3.1). Comparisons among least square means indicated that larvae feeding on ramets that had been naturally defoliated gained significantly less body mass than those from branches of control trees that had been bagged (Fig. 3.2 a). Conversely, the main effect of defoliation did not explain the observed variability in percent mortality of young larvae ($F_{3,125} = 0.86$; $P = 0.46$, Table 3.1).

Three-way ANOVAs of the fourth instar feeding trials suggested that instar duration, relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested (ECD) and

ingested food (ECI) all varied significantly with defoliation treatment (Table 3.2). Recall that these performance measures were based on results from successfully molted and non-molted caterpillars. The non-molted individuals comprised 23% of the total larvae reared on clone 1, 2% on clone 2, and 0% on clones 3 and 4.

Pre-planned pairwise comparisons among the main effect of treatment showed that differences between defoliated and non-defoliated trees only occurred for three of the six performance measures. For instance, larvae which fed on previously defoliated ramets, took significantly longer to complete development of the fourth stadium than those on the control ramets (Fig. 3.2 b). Insects reared on foliage from naturally defoliated trees also had significantly lower RGR than individuals on non-defoliated controls with bags (Fig. 3.2 c). Finally, fourth instars had dramatically lower ECI on the artificially and naturally defoliated ramets as compared to those on the controls (Fig. 3.2 f). None of the pre-planned comparisons between the treatment least square means of RCR were significant following adjustments of the P values with the sequential Bonferroni test (Fig. 3.2 d). The least square means of AD (Fig. 3.2 e) and ECD (not shown) differed significantly only between the bagged and unbagged controls.

Differences among aspen clones accounted for approximately 29% of variance in forest tent caterpillar performance, while the main effect of treatment only accounted for 4%. Percent mortality and biomass gain of first instars that were fed the four different aspen clones varied significantly ($F_{3,125} = 9.67$, $P <$

0.001, and $F_{3,130} = 34.30$, $P < 0.001$, respectively, Table 3.1). Instar development time and nutrition indices of late instars also differed significantly when insects were reared on the various clones (Table 3.2).

Comparisons among least square means indicated that forest tent caterpillars feeding on clone 1 consistently performed more poorly than those from other clones (Figs. 3.3 d, e, g and h). Larvae took at least 1.66 days longer to complete development of the fourth stadium (Fig. 3.3 c) and gained 0.79 mg less biomass (Fig. 3.3 b) than larvae reared on clone 2. This difference became even greater when insect performance of clone 1 was compared to that of clone 4. Caterpillars reared on foliage from clone 1 also had significantly lower growth rates (Fig. 3.3 d), consumption rates (Fig. 3.3 e), and a reduced ability to convert digested (Fig. 3.3 g) and ingested (Fig. 3.3 h) food into biomass than individuals fed from other clones. Mortality of first instars was significantly higher in clone 3, than in any other clone (Fig. 3.3 a). However, most other measures of insect growth and food utilization indicated that insect performance was best or second best on clone 3 (Figs. 3.3 b, c, d, e, g and h). These conflicting results seem to indicate that factors other than leaf quality affected the survivorship of early instar larvae in clone 3. Insects also performed very well on foliage from clone 4 (Fig. 3.3). In general, RGRs, RCRs and other food utilization indices were significantly lower for late instars fed leaves from clone 2 than those fed from clone 3 and 4, but they were still significantly higher than those from clone 1 (Figs. 3.3 d, e, g and h).

Other main effects, such as the canopy level on which first instars fed (i.e., position) did not affect the percent mortality ($F_{2,125} = 0.42$, $P = 0.66$, Table 3.1) or the biomass gain ($F_{2,130} = 1.69$, $P = 0.19$, Table 3.1). Performance also did not vary significantly with sex (Table 3.2). However, the interaction between sex and clone was significant for instar duration ($F_{3,470} = 2.74$, $P = 0.043$, Table 3.2). Males feeding on foliage from clone 1 took 0.61 days longer to develop than females, while the sexes from the other three clones did not differ significantly in this respect. This magnitude of difference between the sexes is unlikely to be biologically significant.

Significant effects on the insect performance measures of first and fourth instars were not only attributed to the main effect of treatment and clone, but also to the interaction between defoliation treatments and clones (Tables 3.1 and 3.2). For this reason, treatment effects must also be discussed with respect to each clone separately.

The manner in which larvae responded to the defoliation treatments varied depending on the clone they had fed upon. Larvae feeding on clone 1 showed the most dramatic negative effects of previous defoliation. Fourth instars from both artificial ($P < 0.001$) and natural ($P < 0.001$) defoliation treatments took 2.28 and 1.22 days longer, respectively, to complete development of the fourth stadium than individuals from the control ramets (Figs. 3.5 a and b). Furthermore, estimates of RGR, RCR, ECD and ECI for larvae fed foliage from defoliated ramets of clone 1 were consistently lower than that of larvae fed foliage from the controls (Figs. 3.6 a, b, 3.7 a, b, 3.9a, b, 3.10 a and b).

Surprisingly, the AD of leaves from the artificially defoliated trees in clone 1 was significantly greater ($P < 0.001$), than for those from non-defoliated trees (Fig. 3.8 a).

In contrast to clone 1, comparisons of insect performance on foliage of the other 3 clones failed to show the same striking differences between defoliated and non-defoliated trees. There were no differences for clone 2. Results for clone 4 indicated that leaf quality decline only slightly in the naturally defoliated trees because larvae reared on these trees had significantly increased RCRs, yet attained similar RGRs to those from the bagged controls (Figs. 3.6 b and 3.7 b). Although first instars from this clone also attained 36% less biomass on trees that were naturally defoliated than larvae from the controls, this effect was non-significant following sequential Bonferroni adjustments of the *P* value (Fig. 3.4 b). Finally, measures of larval performance showed that larvae from clone 3 were more adversely affected by previous defoliation that individuals from clone 4. The RGRs and RCRs for late instar larvae reared on foliage of naturally defoliated ramets were 32% and 20% lower, respectively, than for individuals on the controls (Figs. 3.6 b and 3.7 b).

In general, insect performance measures did not vary between natural and artificial defoliation treatments. Only biomass gain by first instar caterpillars differed significantly between larvae that were reared on artificially and naturally defoliated trees ($P = 0.005$, Fig. 3.2 a). Young larvae fed leaves from the naturally defoliated ramets attained an average body mass that was 44% lower than those fed leaves from the artificially defoliated ramets (Fig. 3.2 a). These

differences could be attributed clearly to the type of defoliation treatment rather than to a "bag effect" because the controls with and without bags were not significantly different ($P = 0.070$, Fig. 3.2 a).

Larvae reared on foliage from the four aspen clones performed variably on the naturally and artificially defoliated ramets. Early instars feeding on clone 4 showed significantly lower biomass gain on insect-damaged trees than on manually-damaged trees ($P < 0.001$, Fig. 3.4 c). The AD for fourth instars also differed significantly between the artificial and natural defoliation treatments of clone 4 ($P < 0.001$, Fig. 3.8 c). However, no significant differences were detected for other larval responses to the two types of defoliation treatments (Figs. 3.5 c, 3.6 c, 3.7 c, 3.9 c, and 3.10 c).

Significant variation in larval performance was also found between artificial and natural defoliation treatments of clone 3. As with clone 4, fourth instar caterpillars had significantly lower RGR when they were fed leaves from the naturally defoliated ramets than larvae fed leaves from the artificially defoliated ramets ($P < 0.001$, Fig. 3.6 c). However, these differences could not be attributed solely to the type of defoliation treatments because the bagged and unbagged controls also varied significantly ($P = 0.002$). Larvae feeding on foliage from naturally defoliated ramets of clone 3 were also 49% less efficient at converting digested and ingested food into biomass than fourth instars feeding on artificially defoliated ramets of clone 3 (Figs. 3.9 c and 3.10 c).

Measures of growth, instar development time and other nutrition indices of early and late instar forest tent caterpillars did not show significant differences

when reared on foliage from the natural and artificial defoliation treatments of clones 1 and 2.

3.5 DISCUSSION

My study is the first to test long-term induced defenses against the forest tent caterpillar, a major defoliator of aspen throughout most of its range. I concluded that long-term induced defenses occurred in trembling aspen a year following severe insect defoliation because larval performance was significantly lower for caterpillars feeding on defoliated ramets than for those on non-defoliated ramets (Fig. 3.2). Other studies have also measured long-term induced defenses in trembling aspen through insect bioassays (Werner 1981, Clausen et al. 1991). However, large aspen tortrix (*Choristoneura conflictana*) larvae were used to assess changes in leaf quality following manual defoliation(s) of trees. Tortrix larvae showed reduced larval weights, reduced pupation success and lower fecundity, as a result of feeding on *P. tremuloides* trees defoliated 100% the previous year (Clausen et al. 1991). Similar results with the same insect species have also been reported in an abstract by Werner (1981).

In marked contrast to the above findings, fall webworm (*Hyphantria cunea*) performance was enhanced on red alder (*Alnus rubra*), in terms of development time and pupal mass, after moderate defoliation by a natural insect population (Williams and Myers 1984). These results, however, must be interpreted

cautiously, because the 'treated' trees (i.e., naturally attacked trees) were not selected randomly, rather herbivores selected them. Thus, their findings may have been confounded by differences in the quality of the foliage prior to the defoliation (for further discussion see Neuvonen and Haukioja 1985). In my study, similar potential problems were averted by carefully recognizing ramets of different clones prior to selecting them for testing of long-term induced defenses.

Leaf quality varied significantly between the four trembling aspen clones (Tables 3.1 and 3.2). Generally, forest tent caterpillars reared on foliage from clone 1 performed most poorly and larvae reared on foliage from clones 3 and 4 performed best (Fig. 3.3). Similar clonal variation in resistance to attack by forest Lepidoptera have previously been reported for a variety of *Populus* species (Chilcote et al. 1992, Robison and Raffa 1994, Hwang and Lindroth 1997). Results in Hwang and Lindroth (1997) are directly comparable to my findings; instar duration, growth and consumption rates of fourth instar *M. disstria* differed significantly, in their study, when they were fed on 13 different trembling aspen clones. Specifically, larvae on their worst clone took 2.4 days longer to complete the fourth stadium and had a RGR 0.75 mg/mg/day lower than those from their best clone. In my study, these differences were 2.1 days between clone 1 and 4, and 1.19 mg/mg/day between clone 1 and 3, respectively (Figs. 3.3 c and d).

In my study, variable performance of larvae on the different clones was likely the result of interclonal variation in aspen's secondary plant metabolites. Levels of 'constitutive' chemical defenses, namely phenolic glycosides and condensed tannins, vary dramatically among trembling aspen clones (Lindroth

and Hwang 1996, Hwang and Lindroth 1997) and elevated levels of these likely provide certain clones a constant level of protection from herbivores (Rhoades 1983b). If constitutive defenses are important in my study, then they are probably genetically based rather than site based because two of the experimental clones (i.e., clones 1 and 2), situated less than 5 meters apart, showed significant differences in leaf quality (see Figs. 3.1 and 3.3).

Trembling aspen clones varied dramatically not only in their resistance to insect defoliators, but also in their responses to previous defoliation (clone x treatment interaction, Tables 3.1 and 3.2). Only clone 1 and 3 showed evidence of induced defensive responses following defoliation. In clone 1, caterpillars reared on previously defoliated trees had significantly longer development times and reduced ECI, but higher AD than those on the control trees (Figs. 3.5 a, b, 3.8 a, and 3.10 a and b). This increase in AD was surprising because less palatable food is usually associated with reduced AD (Scriber and Slansky 1981). The only possible explanation for this was that the lower RCR (Fig. 3.7 a) increased the AD because food was retained in the gut for a much longer period of time (Calow 1977, Scriber and Slansky 1981). In clone 3, growth and consumption rates of fourth instars fed on previously defoliated trees were substantially lower than for those on the control trees (Figs 3.6 b and 3.7 b).

In addressing why trembling aspen clones respond variably to pressures exerted by herbivores, Mattson and Palmer (1988) suggest that clones with high constitutive defenses mount only weak induced defenses because these clones are less likely to be defoliated in the first place, whereas those with low

constitutive defenses have a much higher probability of being attacked and thus are able to mount strong induced defenses. The results from clone 3, in my study, concur with Mattson and Palmer's (1988) findings, whereas those from clone 1 do not. By contrast, my results show that clone 1 had both high constitutive defenses (i.e., larvae had poor performance on this clone), as well as strong induced defenses (i.e., larvae fed from previously defoliated trees performed worse than those from controls). Clones of aspen thus utilize many different strategies against insect herbivores, and their behaviour seems more complex than previously reported.

If prolonged induced changes in leaf quality are specific evolutionary responses to pressures exerted by insect herbivores (Haukioja and Neuvonen 1985, Rhoades 1983b), then it seems appropriate that plants should be able to distinguish between leaf damage from biotic sources, such as a herbivore attack, and abiotic sources, such as a hailstorm or a falling branch (see Baldwin 1988). However, if plants are unable to differentiate between the types of damage then induced defenses are simply passive responses to tissue loss (Williams and Myers 1984). Trees in my study appeared to be induced more strongly by insect feeding than by manual clipping of the leaves. Comparisons of the main effects of treatment, indicated that first instar caterpillars gained 44% less biomass on naturally defoliated trees than those on artificially defoliated trees (Fig. 3.2 a). However, performance measures for fourth instars were not significantly different between larvae reared on manually and naturally defoliated ramets (Figs. 3.2 b to f). There are two ways to account for this. Either early instar caterpillars were

much more sensitive to the subtle differences in the leaf quality of naturally and artificially defoliated trees than were mature larvae (see Lindroth and Bloomer 1991), or qualitative differences between insect damaged and manually clipped trees disappeared as leaves matured (see Scriber and Slansky 1981, Raupp and Denno 1983). Particularly relevant to my overall findings is the study by Haukioja and Neuvonen (1985), who also used bioassays to demonstrate that feeding damage by autumnal moth (*Epirrita autumnata)* on *Betula pubescens* was more effective at triggering long-term induced defenses than was simple tearing of the leaves.

Only one of the two clones that showed induction of defenses also differed significantly between manual and insect defoliation treatments. Fourth instars from clone 3 had significantly lower RGR, ECD and ECI on naturally defoliated trees than larvae on artificially defoliated ones. In clone 1, differences between the defoliation types were not detected. Recall, however, that the natural defoliation treatments from clone 1 and 3 removed less leaf biomass than the artificial defoliation treatments. Clone 3, therefore, appears to require only moderate levels of insect feeding to induce a stronger defensive response than was detected after 75% artificial defoliation. On the other hand, clone 1 may require complete defoliation by an insect herbivore to induce stronger defenses.

The best defense plants have against mobile herbivores is to respond to localized damage through systemic responses that protect the entire plant (Rhoades 1983b). Forest tent caterpillar larvae are highly mobile insects and are not restricted to tents or webs. Even early larval instars, which are more

restricted to their natal tree, often move to new and distant locations on the same tree (Fitzgerald 1995). Results of my experiment indicate that first instar caterpillar performance did not differ significantly between individuals that were reared on previously defoliated and undefoliated branches of the same tree (Table 3.1). This implies that leaf quality of defoliated ramets declined to the same extent throughout the tree. The mobile forest tent caterpillars would then likely move to a less defended plant the following year and thus the previously defoliated plant experiences a reduction in subsequent damage (Schultz 1983). Systemically induced defenses have also been reported for a number of other plant species (Hartley and Firn 1989, Haukioja and Niemelä 1977, Jones et al. 1993, Mutikainen et al. 1996, Wratten et al. 1984).

Results from my study have demonstrated that the feeding damage by forest tent caterpillars is able to induce defensive responses in trembling aspen a year following the initial attack. These responses clearly reduce growth and feeding efficiencies in the larvae, which in turn, likely affect the insect's survival and fecundity (i.e., fitness). For instance, significantly slower rates of development, as was found in my study, prolong the herbivore's exposure to predators, parasitoids and abiotic factors (Rhoades 1983b, Schultz 1983, Parry et al. 1998). Further study of the effects of long-term induced defenses on direct measures of herbivore fitness is needed to more definitively assess the importance of this phenomonen for the population dynamics of *M. disstria*. In addition, long-term studies such as Benz (1974, 1977), Baltensweiler et al. (1977) and Haukioja (1980) are required to determine the number of years that

induced defenses last in trembling aspen, because only the persistence of poor leaf quality causes the negative feedback loop and introduces the time-lags between successive outbreaks of insect defoliators (Benz 1974, 1977, Baltensweiler et al. 1977, Haukioja and Hakala 1975, Haukioja 1990).

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Table 3.1. ANOVA tables for percent mortality and biomass gained by first instar forest tent caterpillars.

		Percent Mortality				Biomass Gained		
3-Way ANOVA	df	MS	*F*	*P*	df	MS	*F*	*P*
Model								
Clone	3	4625.60	9.67	< 0.001	3	13.13	34.30	< 0.001
Treatment	3	412.72	0.86	0.462	3	2.54	6.63	< 0.001
Position	2	200.32	0.42	0.659	2	0.65	1.69	0.189
Clone x Treatment	9	510.84	1.07	0.391	9	0.77	2.02	0.042
Clone x Position	6	485.81	1.02	0.418	6	0.34	0.88	0.514
Treatment x Position	6	425.58	0.89	0.505	6	0.74	1.94	0.078
Clone x Treatment x Position	18	221.82	0.46	0.969	18	0.38	0.99	0.472
Error	125	478.31			130	0.38		

Table 3.2. ANOVA tables for various performance measures of fourth instar forest tent caterpillars.

		Instar Duration			RGR			RCR		
3-Way ANOVA	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Model										
Clone	3	62.66	98.50	< 0.001	23.39	171.14	< 0.001	1259.27	90.40	< 0.001
Treatment	3	7.53	11.84	< 0.001	0.83	6.05	< 0.001	40.18	2.88	0.035
Sex	1	2.28	3.59	0.059	0.03	0.23	0.632	6.19	0.44	0.505
Clone x Treatment	9	5.29	8.32	< 0.001	1.03	7.54	< 0.001	85.18	6.11	< 0.001
Clone x Sex	3	1.74	2.74	0.043	0.02	0.17	0.918	14.61	1.05	0.371
Treatment x Sex	3	0.85	1.33	0.264	0.21	1.53	0.206	18.66	1.34	0.261
Clone x Treatment x Sex	9	0.66	1.03	0.412	0.15	1.10	0.359	6.98	0.50	0.874
Error	470	0.64			0.14			13.93		

		AD			ECD			ECI		
3-Way ANOVA	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Model										
Clone	3	2249.04	34.49	< 0.001	1477.76	54.89	< 0.001	623.46	83.79	< 0.001
Treatment	3	662.90	10.17	< 0.001	247.35	9.19	< 0.001	142.16	19.11	< 0.001
Sex	1	90.49	1.39	0.239	50.83	1.89	0.170	3.95	0.53	0.467
Clone x Treatment	9	288.28	4.42	< 0.001	67.72	2.52	0.008	32.61	4.38	< 0.001
Clone x Sex	3	27.09	0.42	0.742	16.59	0.62	0.605	1.84	0.25	0.864
Treatment x Sex	3	14.74	0.23	0.878	31.99	1.19	0.314	5.35	0.72	0.541
Clone x Treatment x Sex	9	48.42	0.74	0.670	16.52	0.61	0.786	3.70	0.50	0.876
Error	470	65.20			26.92			7.44		

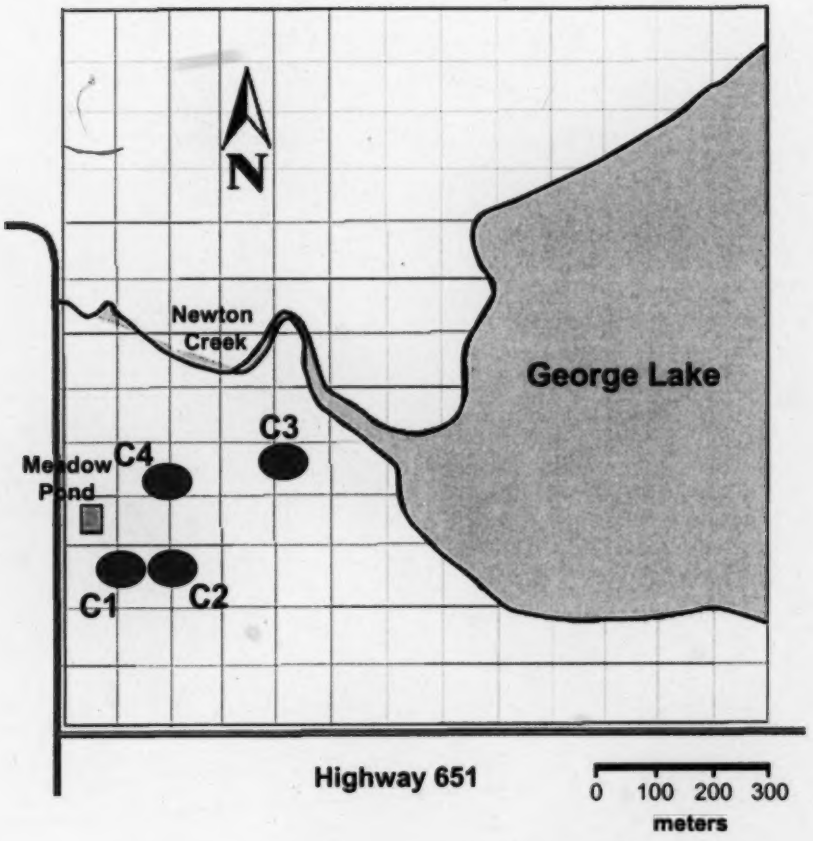

Fig. 3.1. Study location and spacial arrangement of trembling aspen clones.

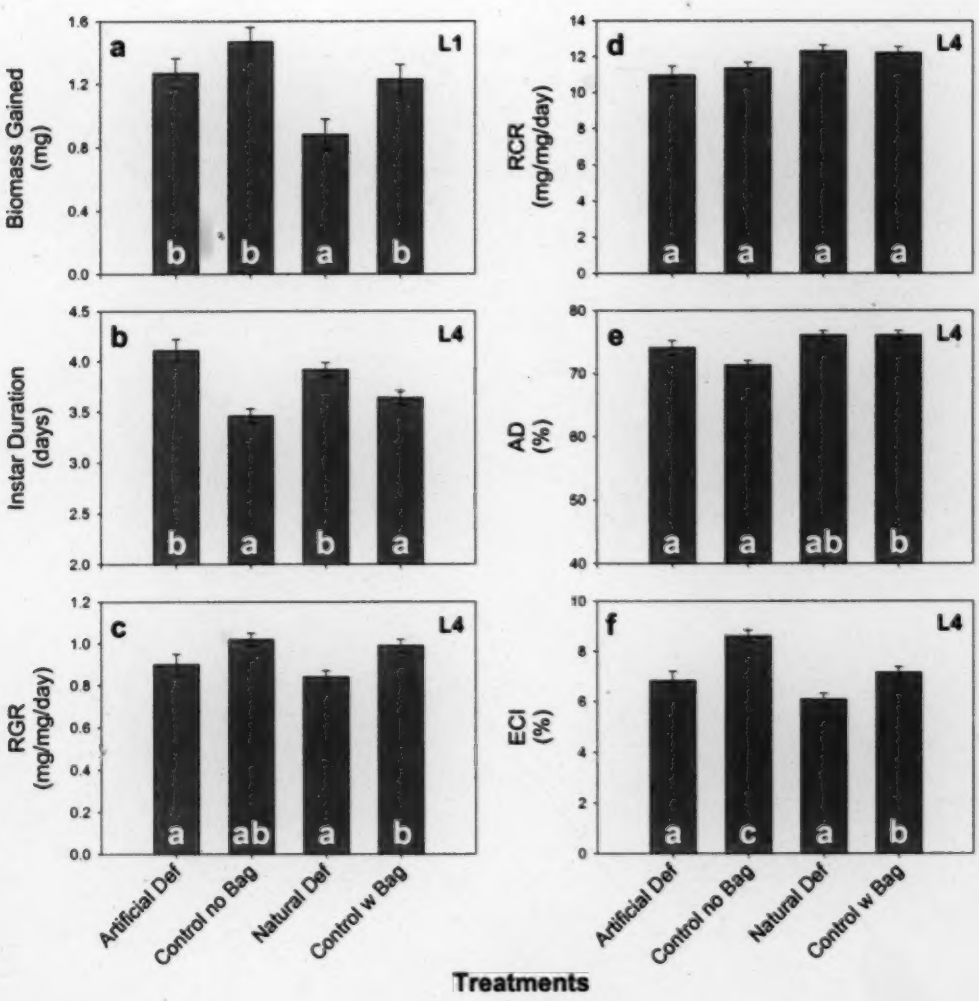

Fig. 3.2. Least square mean (± SE) of biomass gained (a) by first instar larvae (L1) feeding on either artificially or naturally defoliated aspen ramets, or control ramets with or without bags. Least square mean (± SE) of instar duration (b), RGR (c), RCR (d), AD (e), ECI (f) for fourth instar larvae (L4) feeding on the same treatments as the L1s. Bars with the same letter indicate no significant difference.

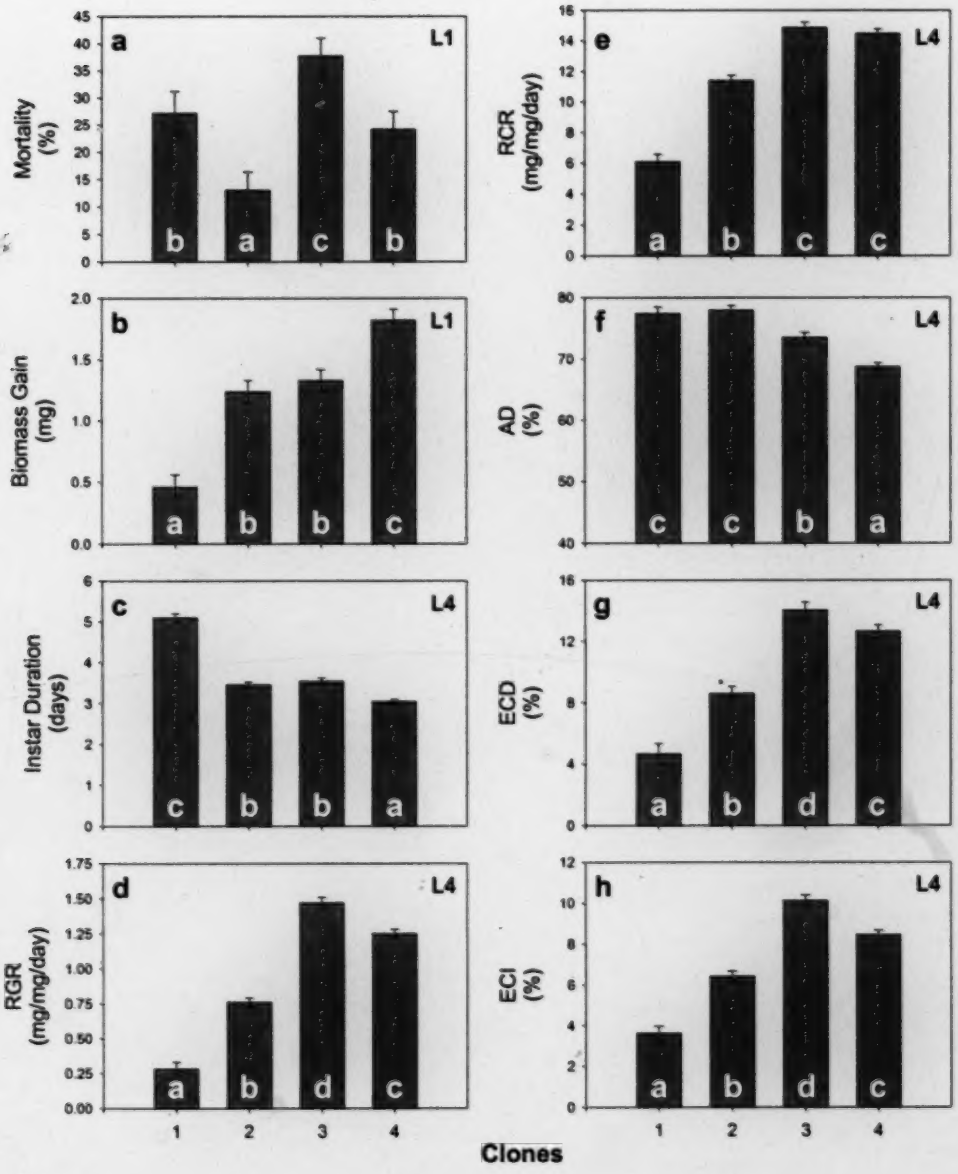

Fig. 3.3. Least square mean (± SE) of percent mortality (a) and biomass gained (b) by first instar larvae (L1) on four clones of trembling aspen. Least square mean (± SE) of instar duration (c), RGR (d), RCR (e), AD (f), ECD (g) and ECI (h) for fourth instar larvae (L4) on the same four aspen clones. Bars with the same letter indicate no significant difference.

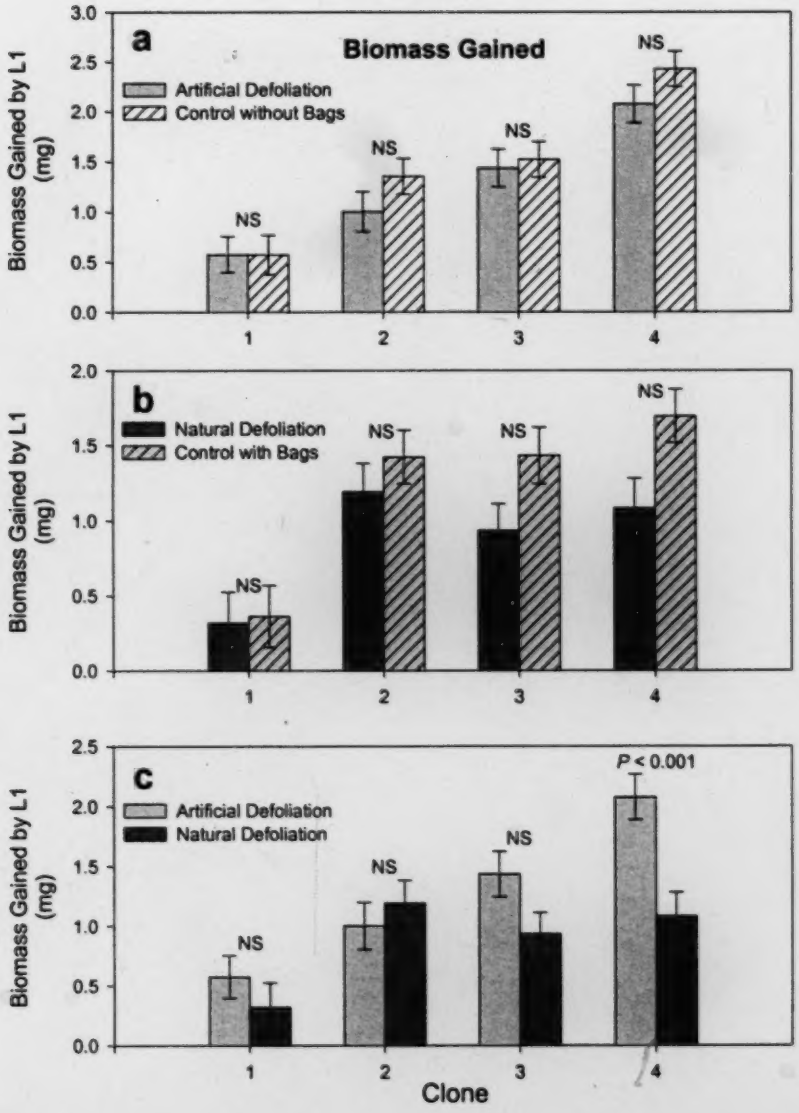

Fig. 3.4. Comparisons of least square means (± SE) of biomass gained by first instar forest tent caterpillars (L1) feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those with NS indicated no significant differences.

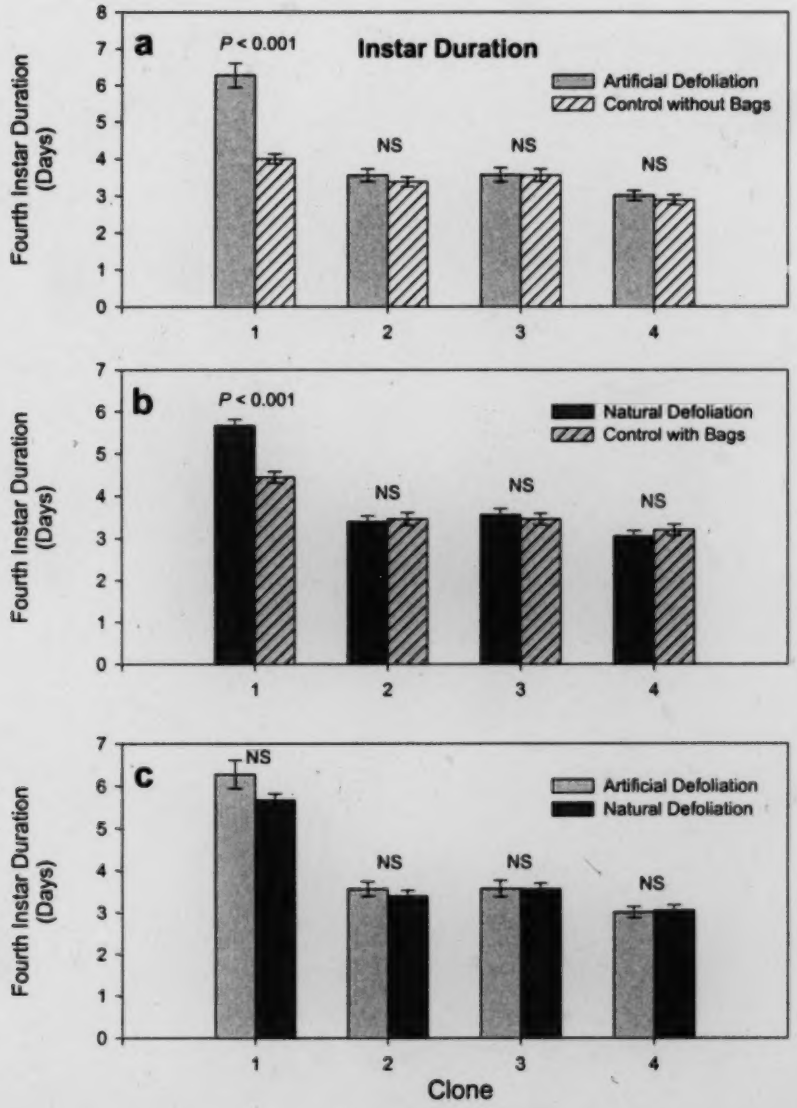

Fig. 3.5. Comparisons of least square means (± SE) of instar duration for fourth instar forest tent caterpillars feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those with NS indicated no significant differences.

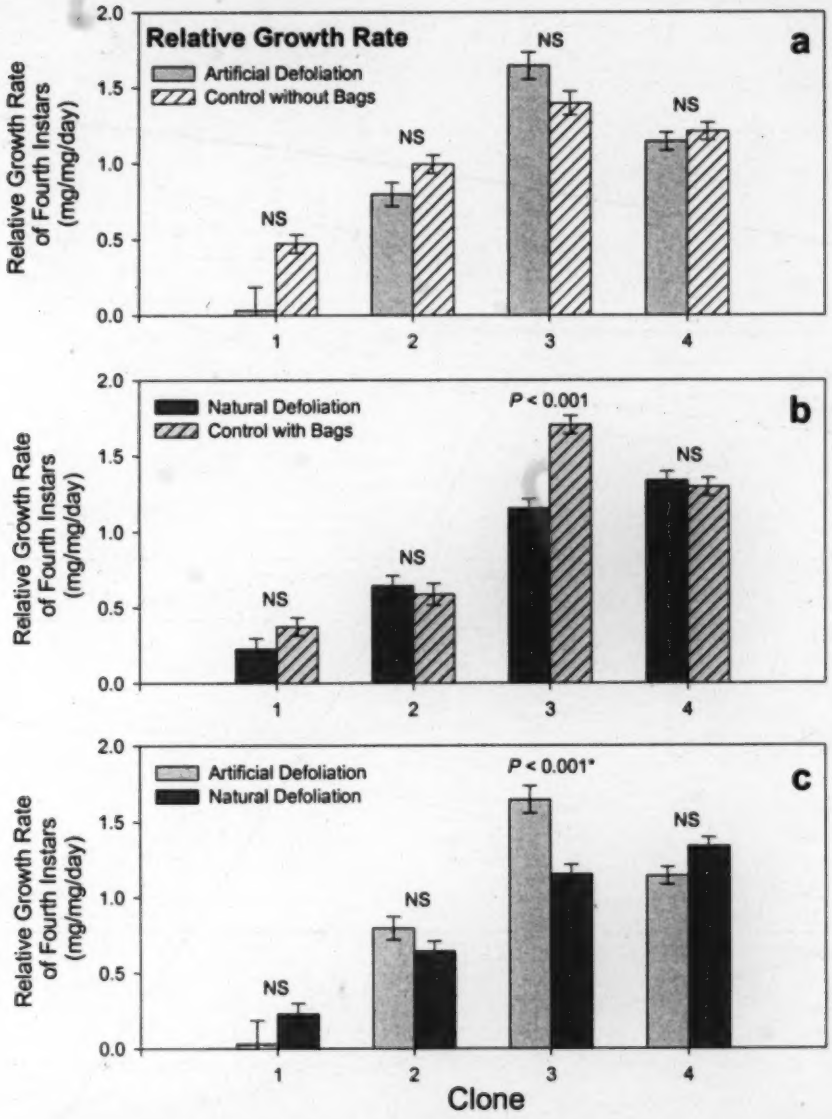

Fig. 3.6. Comparisons of least square means (± SE) of Relative Growth Rate (RGR) of fourth instar forest tent caterpillars feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those followed by * indicate that treatments may be confounded with a "bag effect". Comparisons designated as NS indicated no significant differences.

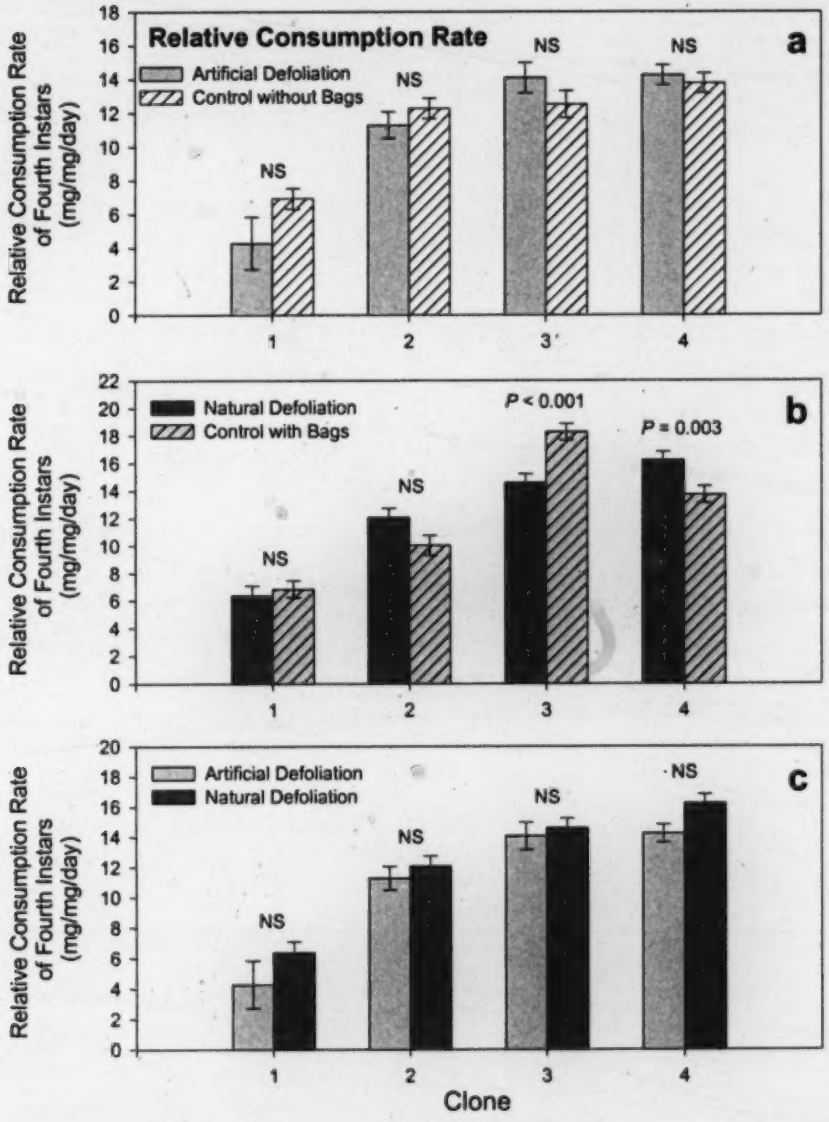

Fig. 3.7. Comparisons of least square means (± SE) of Relative Consumption Rate (RCR) of fourth instar forest tent caterpillars feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those with NS indicated no significant differences.

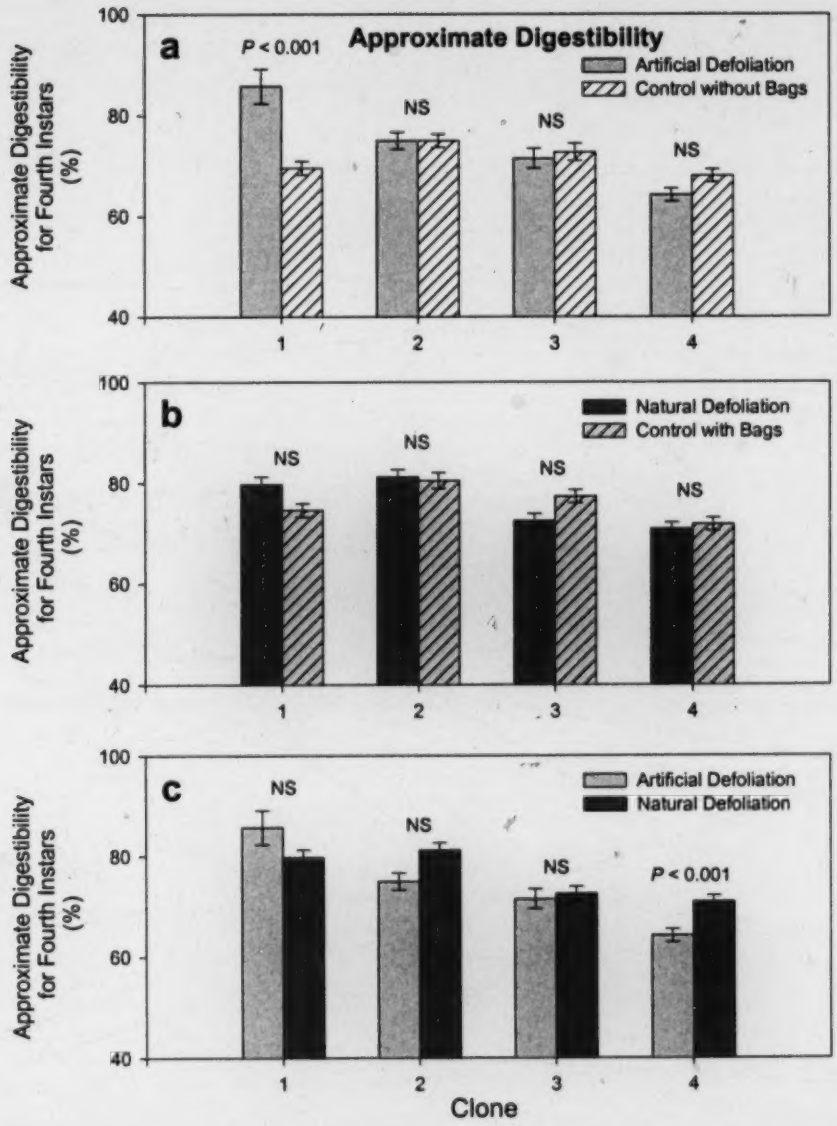

Fig. 3.8. Comparisons of least square means (± SE) of Approximate Digestibility (AD) of fourth instar forest tent caterpillars feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those with NS indicated no significant differences.

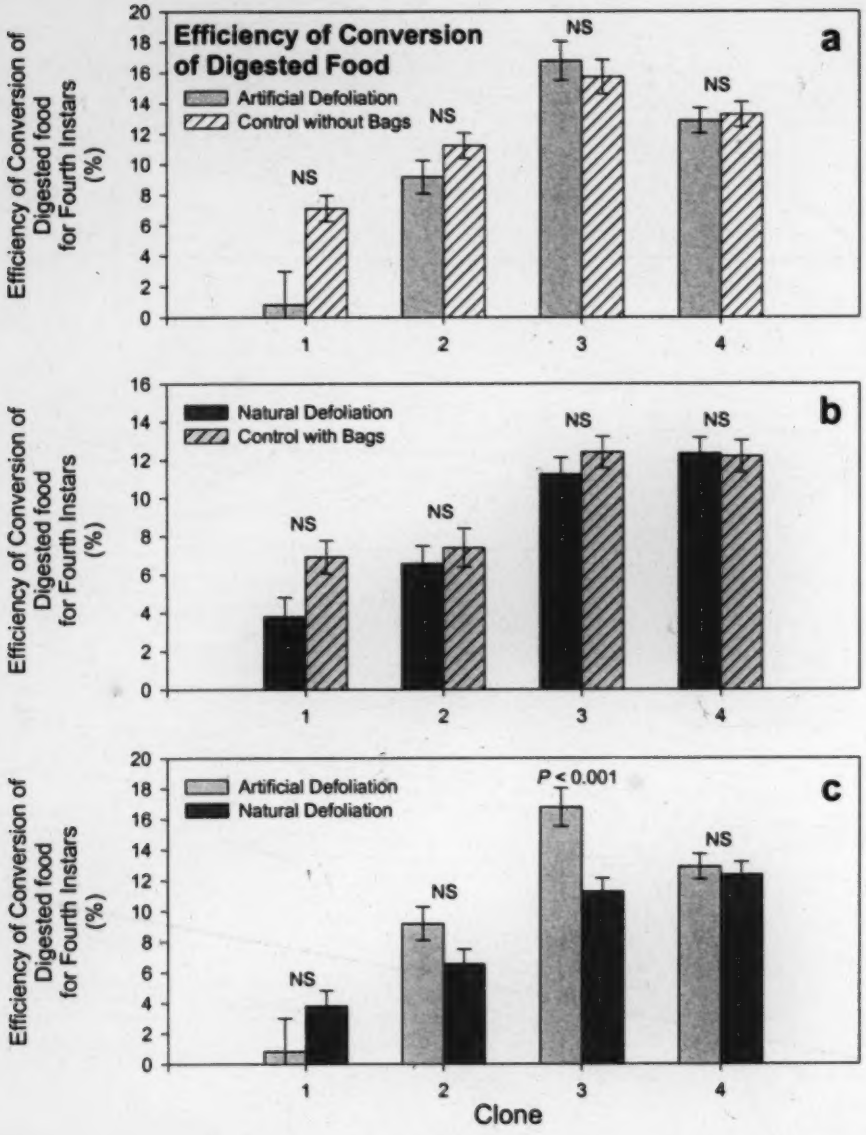

Fig. 3.9. Comparisons of least square means (± SE) of Efficiency of Conversion of Digested food (ECD) of fourth instar forest tent caterpillars feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those with NS indicated no significant differences.

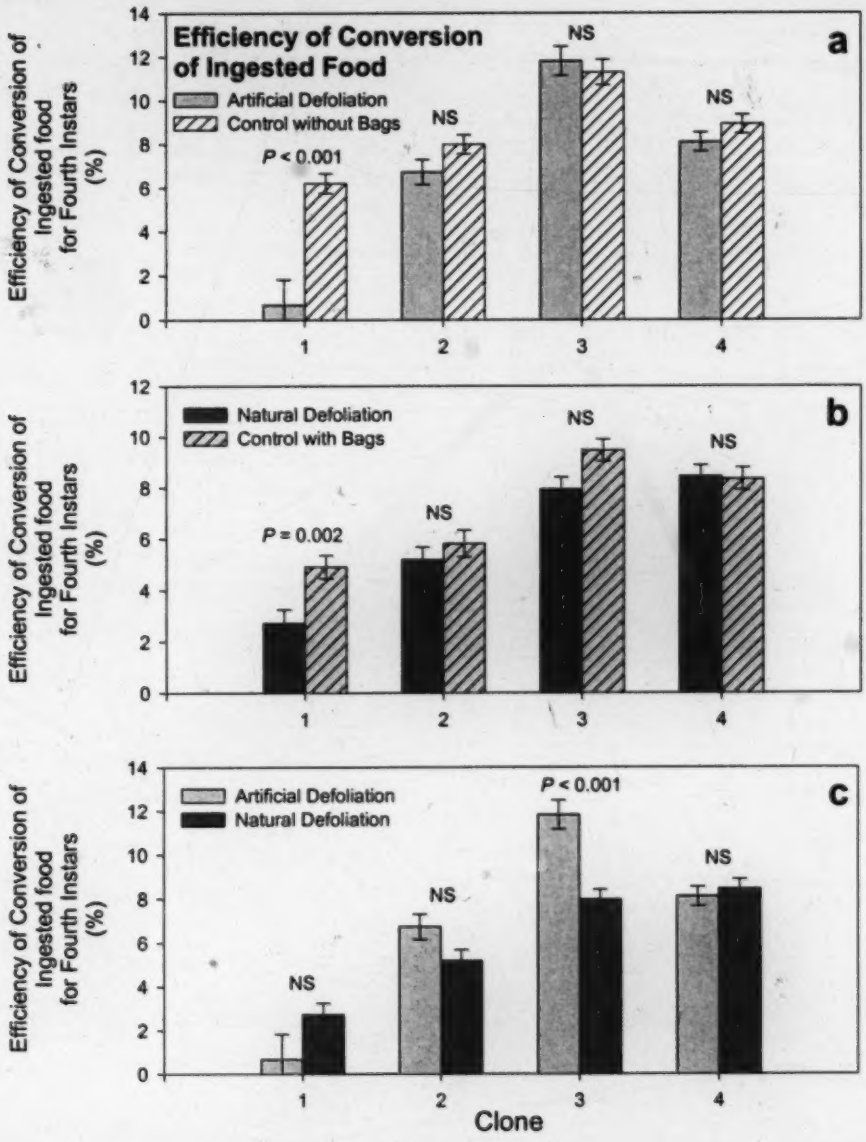

Fig. 3.10. Comparisons of least square means (± SE) of Efficiency of Conversion of Ingested food (ECI) of fourth instar forest tent caterpillars feeding on (a) artificially defoliated ramets (solid coloured bars) and non-defoliated control ramets without bags (diagonally striped bars), (b) naturally defoliated ramets (solid coloured bars) and non-defoliated ramets with bags (diagonally striped bars), and finally (c) artificially and naturally defoliated ramets of four different aspen clones. Comparisons with *P* values indicated significant differences following sequential Bonferroni adjustments, however, those with NS indicated no significant differences.

Chapter 4

Conclusions

4.1 Induced Amelioration

It is known that mammal browsing of dormant winter buds can result in the production of larger, more nutritious leaves in the following growing season (Danell and Huss-Danell 1985, Danell et al. 1985, Senn and Haukioja 1994). This has lead to the speculation that herbivorous insect larvae, which emerge and concentrate their feeding early enough to exploit opening spring buds, could cause a similar response. Senn and Haukioja (1994) have shown experimentally that this is a likely scenario for autumnal moth (*Epirrita autumnata*) larvae feeding on mountain birch (*Betula pubescens*).

In the present thesis, I found no evidence suggesting that forest tent caterpillar (*Malacosoma disstria*) are able to induce such significant positive changes in the leaf quality of its primary host, trembling aspen (*Populus tremuloides*). Instead, leaf quality remained unchanged and even deteriorated to some extent for the caterpillars feeding on previously clipped or mined trees as compared to those of the controls.

Before considering the general applicability of these findings, several factors need to be taken into account. First, is the unknown potential for interspecific variability in this phenomenon; the unique requirements for induced amelioration in aspen may well differ fundamentally from those as already recognized in mountain birch by Senn and Haukioja (1994). Secondly, aspen

being a clonal species, has the potential for high interclonal variability in its response to real and simulated insect damage (Chapter 2 and 3). Therefore, to conclude from a single case study of three clones that all aspen, across the full spectrum of its interclonal variability, is immune to such insect-induced changes is premature. Clones capable of a positive induced response may as yet to be found; wider sampling is clearly required. In a preliminary study on other George Lake clones (Durand and Spence, personal observations), one particular clone appeared to respond positively to apical bud removal (i.e., increased leaf size and chlorophyll content of the remaining leaves).

4.2 Induced Defenses

1. Overall, leaf quality declined dramatically in trembling aspen a year following true and simulated forest tent caterpillar defoliation. As a clear indication of induced defenses, *M. disstria* larvae gained significantly less biomass, took longer to develop and had lower feeding efficiency indices on foliage from defoliated ramets than those reared on foliage from non-defoliated ramets. It remains for future study to determine whether this induced reduction in aspen leaf quality may build up and last for an extended number of years following an outbreak. If this can be shown, long-term induced defenses may well be a contributing factor in explaining the time-lags between peaks of forest tent caterpillar outbreaks (Haukioja and Neuvonen 1987).

To explain the regional synchrony of population fluctuations in forest Lepidoptera, plants (clones) must also respond in a consistent manner to insect attacks (Karban and Myers 1989). Two of my four experimental aspen clones (clone 1 and 3) showed strong induced defenses following one year of severe defoliation. Although responses were not consistent among all clones tested, those failing to show defensive responses may have required two or more years of consecutive defoliation in order to become sufficiently defended. Clausen et al. (1991) found aspen leaf quality to decrease significantly more for large aspen tortrix (*Choristoneura conflictana*) larvae following three consecutive years of 100% defoliation than after one year of such defoliation. The same may well be true of forest tent caterpillar outbreaks, because aspen trees are defoliated over a number of consecutive years in many outbreaks (Duncan and Hodson 1958). Furthermore, the ability of some clones (i.e., clone 1 and 3) to become defended after only one year of forest tent caterpillar defoliation may increase the rate and amount of feeding damage to surrounding non-defended aspen clones (i.e., clone 2 and 4). This may cause these clones to become defensively induced more rapidly than would occur if the defoliation was more evenly distributed throughout the forest.

2. The efficacy of an induced response depends on the spatial extent of that response (Wold and Marquis 1997). Induced responses can be either localized to the area of the plant previously affected by herbivory (Tuomi et al. 1988) or systemic, conferring protection to all parts of the plant, both damaged

and undamaged (Mutikainen et al. 1996). The former case allows a mobile herbivore to simply feed on the remaining unaffected parts of the same plant, thus dispersing the damage, but not necessarily reducing it (Wold and Marquis 1997). In the latter case, however, mobile herbivores would likely move to a less defended plant (Schultz 1983) and thus the previously defoliated plant experiences a reduction in subsequent damage. My data suggest that trembling aspen were well defended against mobile herbivores such as forest tent caterpillars, because leaf quality for larvae decreased in both previously defoliated and non-defoliated branches of the same ramet.

3. Insect damage appeared to elicit stronger induced defenses than simulated wounding. However, I found only first instar caterpillars, not the older fourth instars, were sensitive to the differences in leaf quality of naturally and artificially defoliated trees (see Lindroth and Bloomer 1991). This suggests that induced responses are indeed defensive, having evolved in aspen to deter future insect attacks (Haukioja and Neuvonen 1985). Whether or not they evolved as responses to herbivores in general or are co-evolutionarily linked specifically to *M. disstria* (see De Moraes et al. 1998), remains to be determined.

4. My study is the first to document that 'rapid' induced defenses can occur in aspen the same season, shortly following damage to young, unfurling leaves in the spring (Chapter 2). Ironically, this revealed itself in the amelioration experiment, where, in clone 1, leaf quality declined significantly for larvae fed on

ramets whose buds had been manually clipped earlier in the growing season. Only wounding of fully expanded leaves of aspen has previously been found to rapidly (within 24 hours) induce significant increases in defensive foliar chemicals, that in turn, adversely affected the performance of large aspen tortix larvae (Clausen et al. 1989). Such rapid induced defenses, which act on the herbivores doing the damage, are a stabilizing factor in the population dynamics of the insect (Haukioja and Hanhimäki 1985).

4.3 Importance of host plant quality in tri-trophic interactions

The impact of induced defenses and induced amelioration on current or future population(s) of insect herbivores can only be assessed by some measure of herbivore fitness. Growth rate, survivorship, fecundity or ideally the number of viable offspring must be measured (Rhoades 1979). The fitness of herbivores, as Rhoades (1983) so eloquently states, "should depend upon the nutritional quality of their food plants acting not only through direct effects upon their mortality and fecundity but also through indirect effects upon their susceptibility to parasitism, predation, and disease".

Figure 4.1 illustrates the potential interconnectedness of the several tri-trophic interactions that are likely important in regulating the population dynamics of forest tent caterpillars. Results from my research (indicated by solid lines) are incorporated into this flow diagram to demonstrate that measures of late-instar larval growth and food utilization are good general indicators of future herbivore

success/fitness. The general progression, beginning from the top of the diagram, is as follows:

First, severe defoliation by forest tent caterpillars induces strong defensive responses in ramets in the following year. This potentially reduces the relative consumption rate (RCR), the efficiency of converting digested (ECD) and ingested (ECI) food into biomass for fourth instar larvae. In response to feeding on a systemically induced aspen ramet, the insects' vigor and relative growth rate (RGR) lowers significantly, and this forces the caterpillars to either leave or remain on the tree in which lower foliage quality was induced.

Leaving an induced ramet in search of a more nutritious and/or less defended neighbouring plant, increases the risk of being detected by predators and parasitoids (e.g., Edwards and Wratten 1983, Schultz 1983, Whitham 1981). On the other hand, remaining on the induced tree increases the susceptibilities associated with protracted development time and malnutrition (i.e., reduced vigor, predation, parasitism, disease) (e.g., Parry et al. 1998).

In the end, all factors that either directly or indirectly affect forest tent caterpillar survival and fecundity ultimately reduce the next population of herbivores, and the amount of damage the aspen ramets sustain the next year. The plants, however, may remain induced despite the reduction in herbivory. This results in negative feedback and introduces the time-lags that would explain both the population decline of forest tent caterpillars as well as the long intervals between successive outbreaks. Long-term studies on other important cyclic insect defoliators, namely the autumnal moth (*Epirrita autumnata*) and the larch

budmoth (*Zeiraphera diniana*), have found induced defenses in leaf quality relaxed only after 4 to 5 years of the initial induction (Haukioja 1980, Benz 1974).

4.4 Future research directions

Some of the gaps in our knowledge of interactions between trembling aspen and forest tent caterpillars, and between the caterpillars and their natural enemies are illustrated by the dashed lines in Fig. 4.1. Many of the individual interactions in Fig. 4.1 have been documented to occur with other insect herbivore species, but have yet to studied for *M. disstria*. For instance, Cheng (1970) found winter moth larvae (*Operophtera brumata*) feeding on oak, their preferred host, had higher growth rates and were able to encapsulate a greater proportion of tachinid parasitoids than larvae feeding on alternative hosts. In the same way, this led me to speculate that malnourished forest tent caterpillars feeding on a defensively induced aspen tree may have a greater risk of mortality when the microscopic eggs of an important larval parasitoids like *Leschenaultia exul* (Diptera: Tachinidae) are ingested (Bess 1936) (see boxes from Fig. 4.1 designated, ↓ insect vigor, ↑ susceptibility of parasitoids, and ↓ survival). These exciting possible interactions need to explored in the future.

Little is known about the evolutionary significance of induced defensive responses in trembling aspen. I have shown that aspen possess high interclonal variability in their capacity to mount induced defensive responses. The next step is to determine whether clones that mount strong induced defenses also have

higher evolutionary fitness. As proposed by Karban et al. (1997), "plants with resistance phenotypes that vary as the result of induction are selectively favoured because herbivores experience reduced performance when faced with variability and will thus avoid such plants". If this is true, then adult female *M. disstria* would be expected to be highly selective in their choice of ovipositioning sites on different aspen clones. However, very little is know about this aspect of their life history (Fitzgerald 1995).

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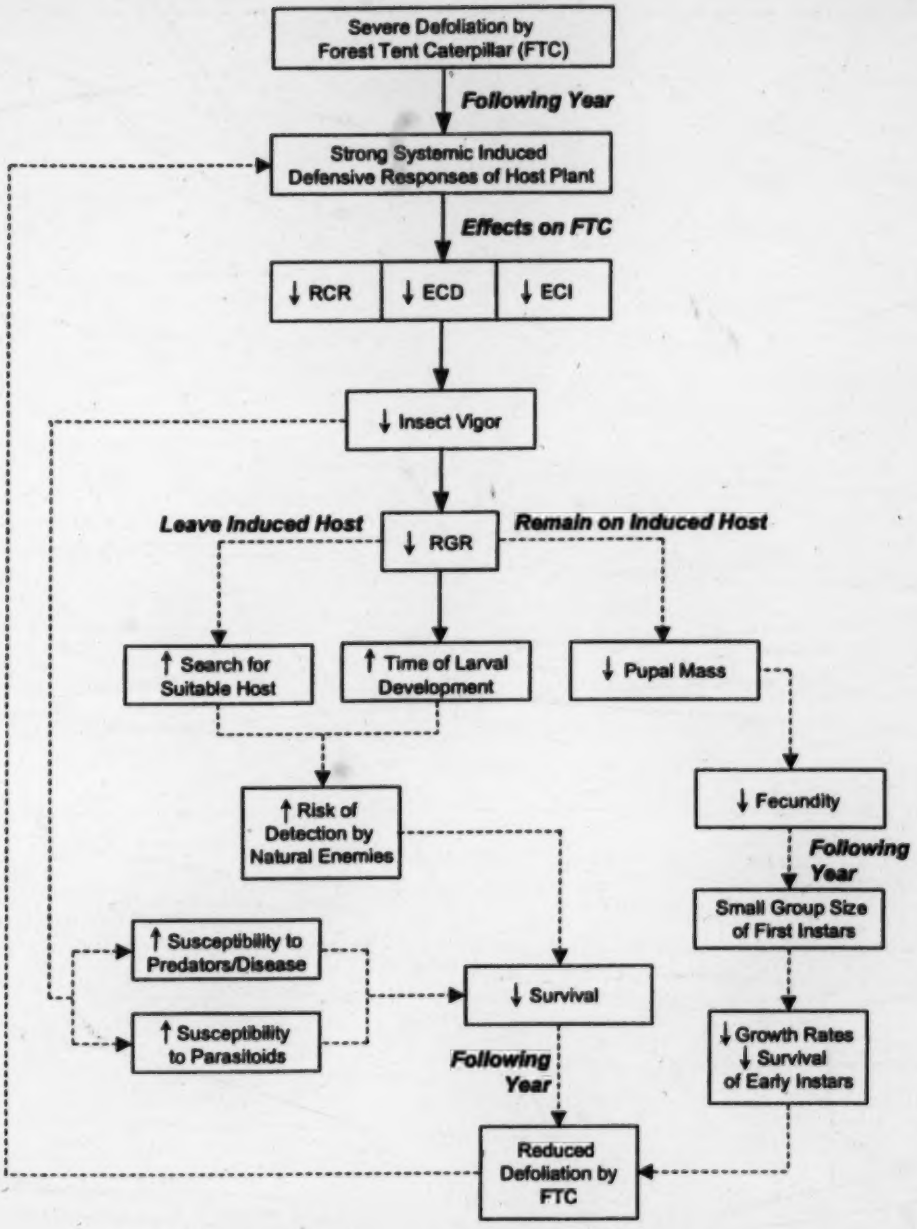

Fig. 4.1. Long-term induced defensive responses of trembling aspen and their direct and indirect effects on forest tent caterpillar (FTC) survival and fecundity. Factors tested in my study are marked by solid lines. Possible factors not yet tested for FTC are marked by dashed lines. See text for further discussion.

Appendix 1. Fourth instar nutritional indices (Scriber and Slansky 1981, Waldbauer 1968).

Relative Growth Rate

RGR = biomass gained / (initial insect weight x time [a])

Relative Consumption Rate

RCR = food ingested / (initial insect weight x time)

Approximate Digestability

AD = [(food ingested - frass) / food ingested] x 100

Efficiency of Conversion of Digested food

ECD = [biomass gained / (food ingested - frass)] x 100

Efficiency of Conversion of Ingested food

ECI = (biomass gained / food ingested) x 100

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[a] time = measured in days and variable for each caterpillar.

Appendix 2.(a) ANOVA table for leaf area to establish which variables significantly affect leaf size.
(b) Relationships between average leaf width and average leaf area for four different aspen clones.

a.

		Leaf Area		
ANOVA	**df**	**MS**	***F***	***P***
Model				
Clone	3	33.89	72.72	< 0.001
Shoot type[a]	1	0.01	0.01	0.921
Length	53	11.17	23.96	< 0.001
Width	53	22.49	48.27	< 0.001
Error	660	0.47		

[a] Shoot type - leaves from either short shoots (1-6 leaves) or long shoots (1-15 leaves)

b.

	Dependent	Indepenent	Regression Statistics					
	Variable	**Variable**	**n**	***F***	***P***	r^2	**Model ***	**C.I.** for *a***
Clone 1	Area	Width	250	2910.5	< 0.001	0.92	$\bar{y} = 9.07 + 4.03\,(\bar{x} - 3.55)$	4.03 ± 0.15
Clone 2	Area	Width	237	2836.9	< 0.001	0.92	$\bar{y} = 15.41 + 5.96\,(\bar{x} - 4.62)$	5.96 ± 0.22
Clone 3	Area	Width	55	508.3	< 0.001	0.91	$\bar{y} = 10.26 + 4.55\,(\bar{x} - 3.77)$	4.55 ± 0.41
Clone 4	Area	Width	235	2408.4	< 0.001	0.91	$\bar{y} = 13.90 + 5.84\,(\bar{x} - 4.24)$	5.84 ± 0.23

* Regression equations for determining average leaf area for each ramet (modified from Cochran 1977, p. 189)
** C.I. Refers to Confidence Interval

Appendix 3. Mean leaf widths and standard errors from various clipped and unclipped ramets of aspen. These averages were used to calculate leaf area.

		Leaf Width		
		n	Mean	SE
	1997			
Clone 1	Manually Clipped (1)	135	3.32	± 0.075
	No Buds Removed (1)	181	3.28	± 0.087
	Manually Clipped (2)	140	2.97	± 0.082
	No Buds Removed (2)	171	2.71	± 0.054
Clone 2	Manually Clipped (1)	123	4.30	± 0.114
	No Buds Removed (1)	0	.	.
	Manually Clipped (2)	109	4.58	± 0.136
	No Buds Removed (2)	181	3.91	± 0.088
Clone 3	Manually Clipped (1)	104	4.03	± 0.101
	No Buds Removed (1)	165	3.87	± 0.074
	Manually Clipped (2)	153	4.13	± 0.092
	No Buds Removed (2)	202	3.76	± 0.065
Clone 4	Manually Clipped (1)	181	3.41	± 0.065
	No Buds Removed (1)	204	3.26	± 0.055
	Manually Clipped (2)	187	3.63	± 0.069
	No Buds Removed (2)	214	3.74	± 0.060
	1998			
Clone 1	Manually Clipped Previous Year (1)	124	3.72	± 0.080
	Manually Clipped (1)	129	3.28	± 0.069
	Mined by Larvae (1)	47	2.97	± 0.146
	No Buds Removed (1)	188	3.26	± 0.060
	Manually Clipped Previous Year (2)	79	2.93	± 0.058
	Manually Clipped (2)	77	3.45	± 0.083
	Mined by Larvae (2)	56	3.17	± 0.108
	No Buds Removed (2)	137	3.13	± 0.060
Clone 3	Manually Clipped Previous Year (1)	49	4.30	± 0.176
	Manually Clipped (1)	118	3.45	± 0.070
	Mined by Larvae (1)	74	3.25	± 0.098
	No Buds Removed (1)	68	4.35	± 0.083
	Manually Clipped Previous Year (2)	63	3.97	± 0.118
	Manually Clipped (2)	59	4.12	± 0.168
	Mined by Larvae (2)	49	3.87	± 0.085
	No Buds Removed (2)	83	4.22	± 0.096
Clone 4	Manually Clipped Previous Year (1)	179	3.89	± 0.068
	Manually Clipped (1)	102	3.91	± 0.114
	Mined by Larvae (1)	88	3.91	± 0.089
	No Buds Removed (1)	77	3.65	± 0.084
	Manually Clipped Previous Year (2)	171	3.81	± 0.071
	Manually Clipped (2)	138	3.66	± 0.078
	Mined by Larvae (2)	87	3.84	± 0.104
	No Buds Removed (2)	162	3.57	± 0.055